gibco

Technical guide to peptones, supplements, and feeds

Enhancing performance of mammalian and microbial bioprocesses

ThermoFisher SCIENTIFIC

Contents

Introduction

The Gibco™ technical guide to peptones, supplements, and feeds was developed as an easy-to-use reference tool to provide technical information and guidance in the selection of performance enhancing products for use in cell culture and microbial fermentation production, from research and development to the final finished product. It is our commitment to innovation and product consistency that makes Gibco™ BioProduction Services a leading supplier and strong global partner. We offer a full line of supplements, feeds, and cell culture media for the biotechnology, biopharmaceutical, and animal and human vaccine markets.

Our dedication to mammalian and microbial media applications is exemplified in our wide range of capabilities:

- **Bioproduction media and supplements manufacturing**—As part of Gibco BioProduction Services, we are focused on the development and manufacture of peptones and supplements. Our full suite of manufacturing services are tailored to meet the needs of your production process—from rapid small-scale medium production to full-scale GMP medium manufacturing. Our comprehensive manufacturing capabilities offer flexibility and capacity without sacrificing quality. Focused on meeting your needs, we are able to customize products, processes, and testing to meet your requirements, all while adhering to stringent quality standards.
- **Full line of supplements and feeds for the biopharmaceutical market**—Gibco BioProduction Services is a pioneer and a trusted partner of leading biopharmaceutical companies around the world. As a result, our peptones and supplements are used in the manufacture of more than 150 animal and human health biopharmaceuticals on the market today. Our offering of animal origin–free, animal origin, and chemically defined supplements and feeds are tailored to work in a variety of biopharmaceutical applications to help you achieve optimal production yields with consistent performance.
- **Media design service**—We offer a full of range of services from the development of custom media and feed formulations to the optimization of existing media and feeds. Our experience, combined with our proven methodology and extensive analytical capabilities, allows us to provide customers with media options that work with their unique specifications, with time and budget in mind.

History

Beginning in 1895, Difco Laboratories produced high-quality enzymes, dehydrated tissues, and glandular products to aid in the digestion process. Meat and other protein digests were developed to stimulate the growth of bacteria and fungi. Extensive research led to the development of Difco™ Bacto™ Peptone, which was introduced in 1914. Building on this knowledge base, we have continued to develop and expand our peptone offerings under these premium brands to support the wide range of requirements and applications for the bioproduction market.

In 1997, Becton, Dickinson and Company (BD) acquired Difco Laboratories and, in 2006, created the Advanced Bioprocessing product line that focused on high-quality solutions to meet the growing needs of the biopharmaceutical market.

Today, Thermo Fisher Scientific offers the Advanced Bioprocessing products after acquiring them from BD in 2018. This integration combines the historic, trusted Difco and Gibco premier brands and provides comprehensive supplements and media solutions in one portfolio, delivering even greater value to our customers.

Service

The Gibco BioProduction Services network maintains inventory throughout the world. With multiple manufacturing locations and a global distributor network, we are prepared to provide a variety of solutions to support customers' needs. For questions or more information on products and services not listed in this guide, please contact your Gibco BioProduction Services representative. To find out more about our proven portfolio of solutions, go to our website at **thermofisher.com/advbio**.

From protein to peptones

For more than a century, peptones have played a central role in the development of high-performance media and feeds spanning the diagnostic, bioproduction, and vaccine industries focused on both human and animal health. Bacto Peptone, the first commercialized peptone, was developed by Difco Laboratories in 1914 and was considered the premium quality standard of supplements for microbial growth media. Building on this knowledge base, Difco continued to develop more peptones to add to the Bacto line of products. Bacto™ Proteose Peptone, Bacto™ Proteose Peptone No. 2, and Bacto™ Proteose Peptone No. 3 were created based upon data indicating that no single peptone was the most suitable nitrogen source for growing fastidious bacteria and supplementing culture media. Peptone development expanded throughout the 20th century to meet the evolving needs within the microbiology, molecular biology, biotechnology, and genetics fields.

As early biotherapeutics were being developed, animal origin (AO) peptones were identified as ideal replacements for serum in mammalian bioproduction processes and began to be routinely incorporated into cell culture media and feeds. During this time, we began to lead the way in developing high-quality peptones designed to meet the unique requirements of the bioproduction industry. When transmissible spongiform encephalopathies (TSE) and bovine spongiform encephalopathy (BSE) risk reduction became a high priority, we began to introduce a number of Difco™ animal origin–free peptones designed to provide the same high level of performance as animal origin peptones. For processes that require chemically defined (CD) materials, we also offer a variety of high-performance CD supplements and feeds. Our premium peptones and supplements are used in a variety of applications and are currently used in the manufacture of more than 150 biopharmaceuticals in the human and animal health markets. We continue to innovate and develop new supplements to meet the increasingly demanding and evolving world of biotechnology.

What are peptones and how are they made?

Peptones, also known as protein hydrolysates, are the water-soluble products derived from the partial hydrolysis of proteins from plant, yeast, or animal sources. The protein hydrolysis process can be accomplished using strong acids, bases, or proteolytic enzymes, which create a complex, nutritionally enriched final product that is easily used by cells. Figure 1 shows the generic composition of a typical peptone to highlight the diverse nutritional profile that is achieved through the hydrolysis process.

While most peptones have similar component groups, the hydrolysis process and starting protein material result in unique product composition profiles, which underlies the existence of multiple products made from the same source material. Acid or base hydrolysis processes, generally carried out at high temperatures, attack all peptide bonds and can destroy some individual amino acids while converting others to their acidic forms.

This process can also destroy vitamins and increase the overall product salt content. Proteolytic enzymes, including microbial proteases, usually result in a gentler process that is run at lower temperatures and can target specific peptide bonds.

Regardless of the digestion method, most peptones are manufactured using a process similar to the diagram shown in Figure 2. Protein and demineralized water are combined to form a thick suspension of protein material in large-capacity digestion vessels, which are stirred continuously throughout the hydrolysis process. For acid hydrolysis, the temperature is adjusted and the digestion material added to the vessel. For proteolytic digestion, the protein suspension is adjusted to the optimal pH and temperature for the specific enzyme chosen for the hydrolysis. The desired degree of hydrolysis depends on the amount of enzyme, time for digestion, and control of pH and temperature.

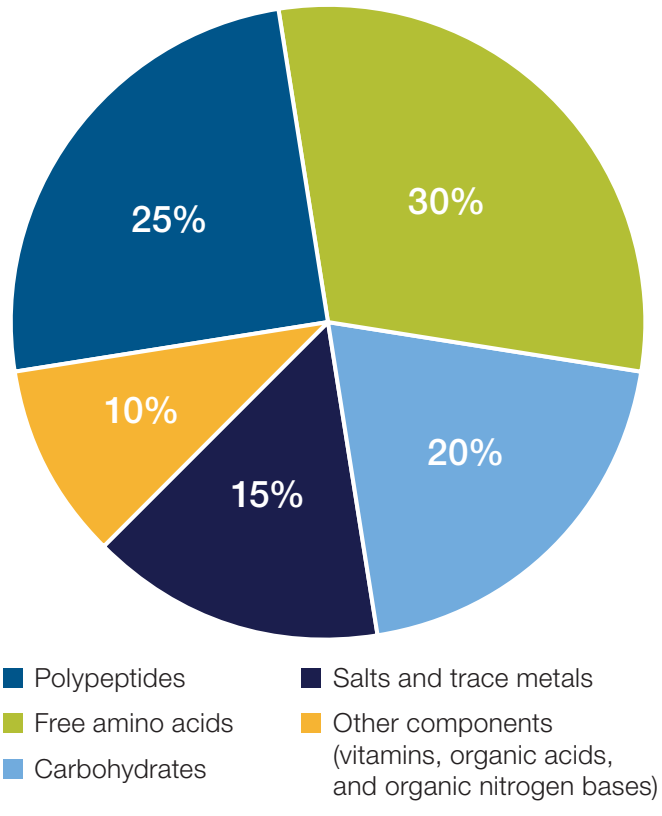

Figure 1. Components of a typical peptone. The data in this chart are based upon the average values for a variety of peptones. They do not represent any specific peptone and are meant to provide general guidance on composition.

Once the predetermined degree of protein digestion is achieved, the activity must be halted; the suspension is either heated to inactivate enzymes or neutralized to inactivate acids or bases. The protein slurry is then centrifuged and/or filtered to remove insoluble materials and to clarify and concentrate the product. Vacuum-evaporation may be used for rapid concentration. This peptone syrup may then undergo further processing for pH adjustment, pasteurization, and/or filtration. Peptones designated with "UF" in their name have undergone an additional ultrafiltration step using a molecular weight cut off (MWCO) membrane to remove larger molecular weight materials including some fats, polypeptides, or protein material. The final drying step of the process further concentrates the peptone by spray drying or by pan drying in vacuum ovens, which readies the material for packaging.

Yeast extract manufacturing is unique because the hydrolysis is done through autolysis of *Saccharomyces cerevisiae*. The yeast is grown to a high cell density in a molasses-based medium optimized for the particular yeast strain and the batch culture is exposed to a controlled temperature or osmotic shock that causes the yeast to die without inactivating the yeast's endogenous enzymes. This begins the autolysis, where the yeast's own digestive enzymes are responsible for breaking down the yeast protein. Once autolysis is halted, insoluble material is separated out by centrifugation and several filtration steps [1]. The final product is then concentrated, ultra-filtered if required for the specific product, and spray dried to ready the material for packaging.

Gibco peptones are produced using the high standards expected from the biopharmaceutical industry. Our raw materials and manufacturing conditions for protein hydrolysis are controlled to produce consistent peptone products. Ingredients used for peptone manufacture, including the protein, agent of hydrolysis, and any buffering agents, are selected based on specific purity and quality standards. The conditions of the hydrolysis, such as the amount of enzyme used, the time for digestion, and the pH and temperature at which hydrolysis is conducted, determine the degree of hydrolysis and the quality of the hydrolysate. Therefore, these conditions are carefully controlled throughout the manufacturing process. Purification, concentration, and drying steps are carefully regulated due to their bearing on the characteristics of a peptone. Finally, each batch of peptone is tested for an array of physical, chemical, analytical, and growth support tests to ensure product quality and lot-to-lot consistency.

What are the benefits of using peptones?

Peptones have a long, proven history of being used to create robust, high-performance media and supplements. Given the unique nature of peptones, they are able to provide numerous benefits to the culture with the addition of just one supplement. As shown in Figure 1, peptones have a diverse nutritional profile that can provide protective effects to the cells, including nutritional buffering, and protection from toxic component levels from the media or process, allowing for the addition of high concentrations of key components, and delay of apoptosis.

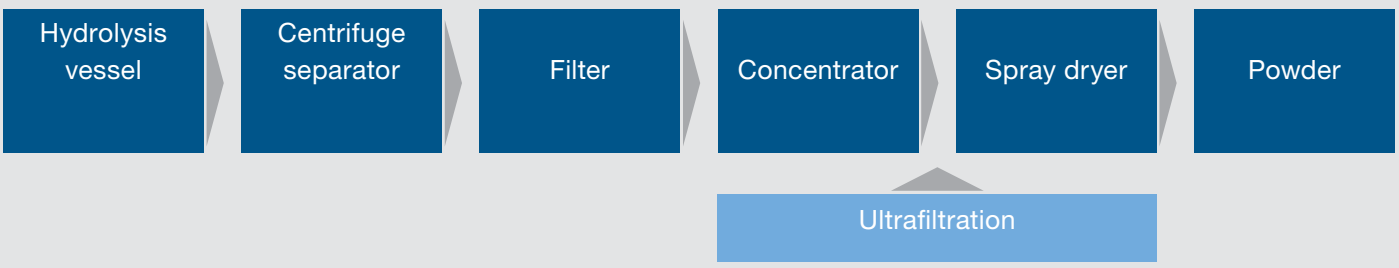

Figure 2. Basic steps of peptone manufacture.

Cell lines, media, and process all contribute to culture performance, so adding the right peptone can dramatically improve the culture environment to achieve production goals.

Peptones are also versatile and can be used with a wide variety of mammalian and microbial cell types to support high growth and protein production. This is demonstrated in Figures 3, 4, and 5 where the same Gibco peptones were tested with each organism. For *S. cerevisiae* and *E. coli*, the peptones were added to M9 salts with glucose and compared to tryptic soy broth, which is a general microbial growth medium. The same peptones were evaluated with a CHOK1 cell line using 5 g/L of each added to a CD, complete medium. All of the peptones increased performance in all three cell lines, thereby demonstrating the versatility of peptones as a quick and effective performance enhancer.

Since peptones are so nutritionally rich, they can be used to quickly optimize media and feeds to achieve high cell growth and titer with minimal resources. They can be added to the base medium, or used as a feed either individually or in combination with other feeds. While individual peptones enhance performance, blending peptones can create synergistic effects that result in higher performance than with either peptone used alone. Peptones can also be used to modulate protein quality to achieve and maintain the desired product profile. All of these attributes make peptones a valuable tool for creating a consistent, high-performance process.

How do I pick the right peptone for my process?

Given the wide variety of peptones that are available, it is important to identify and focus testing on those products that have been specifically developed for your application. Process consistency is critical, so understanding and controlling variability is essential to developing a robust process. Process variability can come from many sources, including the media and supplements used. All raw materials, whether they are peptones or CD components, have inherent variability that can contribute to unacceptable levels of process variability resulting in missed production targets. Regardless of your process or raw material source, it is important to characterize the key drivers within your process that affect culture performance. Once these key drivers are identified, they can be controlled and monitored to drive process consistency and meet production goals.

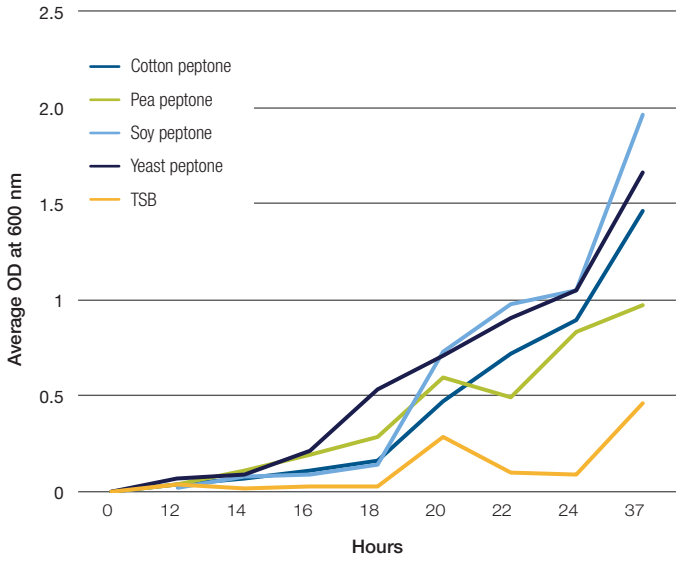

Figure 3. Performance comparison of different peptones across *S. cerevisiae*.

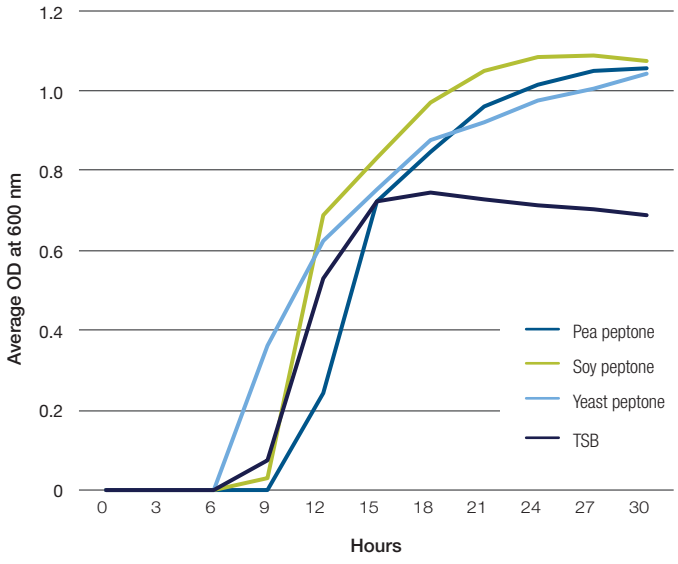

Figure 4. Performance comparison of different peptones across *E. coli*.

To ensure the best peptone or peptones are identified for your process, it is critical to perform a comprehensive screen of different products to ensure the optimal conditions are selected. To help simplify the peptone screening process, several factors can be considered to help narrow the number of products to include in the study.

For mammalian cells, it is important to start the screening process as early in the optimization process as possible so that the peptone is selected and optimized along with the base medium. While any medium can be enhanced with peptone additions, more enriched base media generally yield better results than deficient media. It is important to screen a wide variety of peptones from multiple sources, as well as multiple products from the same source, since they will have different nutritional profiles. A thorough experimental design should be conducted where each peptone is assessed at multiple concentrations, both individually and blended with other products. During these evaluations, it is critical to evaluate all key performance criteria such as growth, production, and protein quality profile to ensure acceptable performance is achieved. Once peptones are identified, it is critical to characterize the culture to establish the baseline of performance and identify key process drivers. Refer to the "Cell culture applications" and "Protocols and definition of methods" sections of this guide for more information on how to select the best peptone for your application.

For optimal growth, microorganisms generally need available sources of carbon, nitrogen, inorganic phosphate and sulfur, trace metals, and vitamins, which are readily available in most peptones. Peptones for microbial applications act as the major source of nutrition and buffering within the culture, and are generally used at concentrations up to 30 g/L. Since they are such a critical component of microbial media, screening must be done to identify which peptones will meet the unique needs of the particular organism. For example, anaerobes and aerobes have different nutritional preferences based upon their oxygenation requirements. Typical analysis data provided for each product in this guide can help to identify products which have compositions that might meet the culture needs.

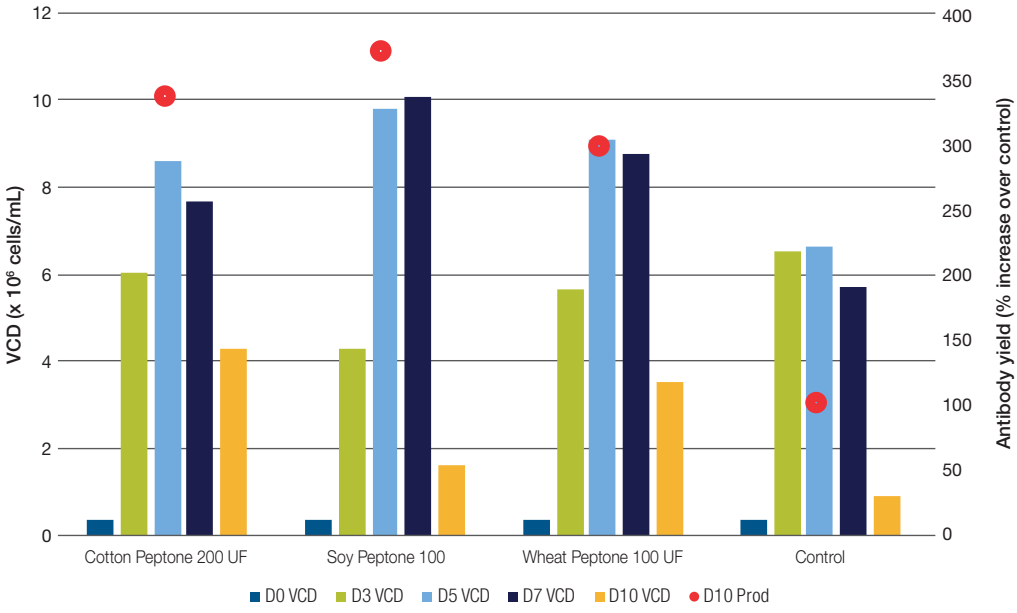

Figure 5. Performance comparison of different Gibco™ peptones across CHO K1 cell line. D: day; VCD: viable cell density; Prod: production.

These products should be tested in a minimal basal medium with an additional carbon source such as glucose, glycerol, or methanol. Some microorganisms prefer blends of peptones, so it is ideal to execute a study where both are evaluated. It is important to evaluate key process parameters to be sure that the peptone is meeting all production targets. Once peptones are identified, it is critical to characterize the culture to establish the baseline of performance and identify key process drivers so they can be monitored and controlled. Refer to the “Fermentation applications”, “Microbial and vaccine media”, and “Protocols and definition of methods” sections for more information on how to select the best peptone or peptone-based medium for your application.

Are there chemically defined peptones?

While there are no CD peptones, CD supplements and feeds provide a viable alternative. CD supplements can be used with either mammalian or microbial cultures to boost the performance of culture media by providing additional key nutrients required by the cells during the initiation of the production. CD feeds are generally used with mammalian cultures and are added throughout the culture life to provide key nutrients for optimal culture performance. Regardless of your application or need, we have a product that can help to meet your production goals. Refer to the “Chemically defined supplements and feeds” and “Protocols and definition of methods” sections for more information on how to select the best CD supplement or feed based upon your application.

Reference

1. Sommer. 1998. Yeast extract: production, properties and components. *Food Aust.* 50:181-183.

Cell culture applications

Introduction

In the biopharmaceutical industry, the requirement for high-performance processes has prompted a greater focus on cell culture media and process optimization [1]. As a result, improvements in cell culture media, supplements, and feeds have enabled significant advancements in bioproduction titers. While sub–gram per liter titers were once the norm, multi–gram per liter titers are now common, with some exceeding 10 grams per liter. While increasing and maintaining protein titer continues to be a key bioprocessing goal, ensuring acceptable protein quality has become an equally, if not greater, critical process specification. This is especially relevant to the ever-increasing number of biosimilar therapeutics being developed. It is desirable to achieve a cost-effective level of production, and it is essential that the quality characteristics of biosimilars, such as glycosylation and charge variant profiles, match those of originator molecules. Monitoring product quality, while optimizing media, supplements, and feed strategies, is essential to developing a process that results in the desired target molecule.

When optimizing a medium or feed, both peptones and chemically defined (CD) supplements should be considered, since they can quickly and cost-effectively improve cell performance. CD media and feeds presumably provide higher levels of raw material consistency and reduced risk from the lack of animal origin (AO) or CD raw materials. Although not completely defined, peptones are well-characterized and long-established cell culture supplements and feeds which can enhance cell growth and/or protein titer while helping achieve or maintain desired protein quality.

Table 1. Gibco™ peptones for cell culture.

Product name	Substrate	Applications
Bacto Yeast Extract	Yeast	Good for growth promotion, especially with insect cells
Yeast Extract	Yeast	Used for growth promotion and protein production
Bacto Yeast Extract Technical	Yeast	Used for growth promotion and protein production
Difco Yeast Extract UF	Yeast	An ultra-filtered yeast extract with an endotoxin limit of 500 EU/g
Bacto TC Yeastolate	Yeast	Good for growth promotion, especially with insect cells
Difco TC Yeastolate UF	Yeast	An ultra-filtered version of Bacto TC Yeastolate with an endotoxin high limit of 500 EU/g
Bacto Malt Extract	Barley	Used for growth promotion and protein production
Phytone Peptone	Soy	Used for growth promotion and protein production, as well as a good, animal origin–free alternative to serum
Difco Phytone Supplement UF	Soy	An ultra-filtered version of Phytone with an endotoxin limit of 500 EU/g
Difco Soytone	Soy	Used for growth promotion and protein production
Bacto Soytone	Soy	Used for growth promotion and protein production, uses an animal-based enzyme in the digestion of soy flour
Soy Peptone 100	Soy	Used for growth promotion and protein production
Wheat Peptone 100 UF	Wheat	Used for growth promotion and protein production
Cotton Peptone 200 UF	Cotton	Used for growth promotion and protein production
Bacto Proteose Peptone No. 3	Porcine	Used for growth promotion and protein production, as well as a good alternative to serum
Bacto Tryptose	Bovine (milk), Tissue, Porcine	Used for serum-free supplement for human diploid fibroblasts
Bacto TC Lactalbumin Hydrolysate	Bovine (milk), Porcine	Used for amino acid supplementation
Difco TC Yeastolate UF	Yeast	An ultra-filtered version of Bacto TC Yeastolate with an endotoxin high limit of 500 EU/g
Bacto Proteose Peptone No. 2	Porcine	Used for growth promotion and protein production

Peptones are a rich source of amino acids, peptides, vitamins, carbohydrates, nucleosides, minerals, and other components. Therefore, peptones can serve as an optimal nutritional source for cell culture. Peptones have also been shown to provide additional benefits to cell culture, including exhibiting anti-apoptotic effects [2,3] and positively affecting cell cycle [4] and/or cell metabolism [5]. The rich composition of peptones can be an advantage by providing nutritional and environmental support to the culture.

Due to their defined nature, CD supplements are a desirable option, but their use requires much tighter control over component concentrations during the life of the culture. Multiple instances have been documented where the selected components influenced the performance of CD media formulations. For example, limiting levels of tyrosine in CD media has been shown to result in monoclonal antibody (mAb) sequence variants in Chinese hamster ovary (CHO) cells [6]. Different forms of iron have been shown to differentially affect recombinant protein glycosylation [7], and the concentration of copper in cell culture media can directly impact mAb charge variant species [8]. Unsuspected variability in CD raw materials has also been found to impact protein quality. For example, variable amounts of the trace metal manganese, present as an impurity in ferrous sulfate, have been shown to result in a shift in the mAb glycosylation profile [9]. Similarly, various trace element contaminants in different lots of salts and amino acids have contributed to changes in titers and product quality [10]. This has led to the need for increased transparency and consistency in the source and supply chain of critical CD raw materials.

Genomic, proteomic, and metabolomic heterogeneity among cell types and cell lines results in each individual cell line having its own distinct performance attributes and nutritional requirements. Therefore, it is critical to identify a supplement that will appropriately meet the unique needs of a specific cell line. When choosing a peptone or CD supplement to enhance cell culture performance, it is critical to understand the key drivers of each individual process and appropriately match the supplement to the cell line and process. Only a methodical strategy can determine the base medium, peptone or CD supplement, and feed strategy that will yield the greatest benefit.

Base medium selection

The ideal base medium is one that requires little to no cellular adaptation and can maintain acceptable viable cell numbers for an extended period of time while achieving the targeted protein titer. Although there are many viable commercially available media options, none are designed to meet the specific requirements of a particular cell line without further optimization. Base medium selection is the critical starting point of the optimization process, so it is essential to identify the proper medium prior to initiating supplementation. Classical media, such as RPMI and DMEM, do show increased performance with the addition of peptone or CD supplementation. However, complete formulations designed for biopharmaceutical production perform the best and are the preferred starting point for optimization. Production formulations vary in their composition and can result in varying levels of performance in different cell lines, as shown in Figure 1. Protein titers in two different CHO cell lines vary across five base media. This demonstrates the importance of evaluating multiple base media to determine which performs best with the specific cell line in use.

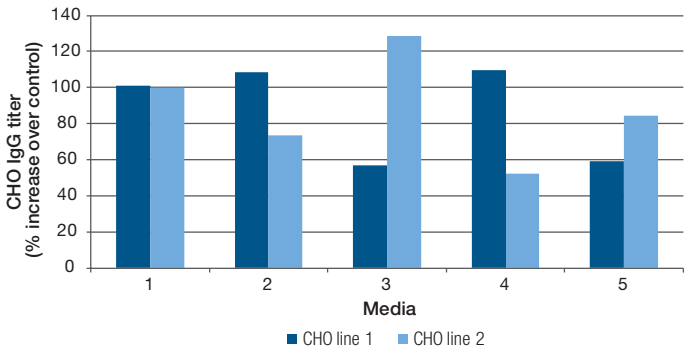

Figure 1. Performance comparison of CHO media.

Supplement and feed selection

The benefits of supplements and feeds in cell culture applications have been well documented for many years. Since every supplement and feed is different and each cell line has unique nutritional requirements, it is critical to evaluate a wide range of peptones or CD supplements to identify the ones that work best for a particular cell line and production process. Even different peptones produced using the same protein source should be considered since their different digestion profiles can change the overall composition of each product.

To run the most effective screen, the list of potential peptones should be narrowed to include only the candidates best suited for the specific process being developed. For example, if endotoxin levels are a concern, limit the screen to ultra-filtered (UF) peptones. If the cell line utilizes the glutamate synthetase (GS) expression system, wheat peptones might be excluded from the screen due to their glutamine-rich composition. Also, consider regulatory requirements and release criteria, since these factors vary between manufacturers. Multiple peptones should be evaluated to determine those that deliver the best performance with the base medium of choice and the cell line.

Although fully defined, CD supplement composition can vary considerably from one supplement to the next. Multiple CD supplements should be screened to increase the likelihood of identifying the optimal pair of supplement and base medium.

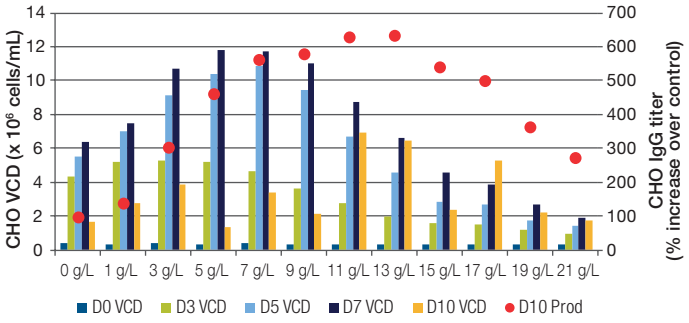

Figure 2. Peptone titration in a complete medium. D: day; VCD: viable cell density; Prod: production.

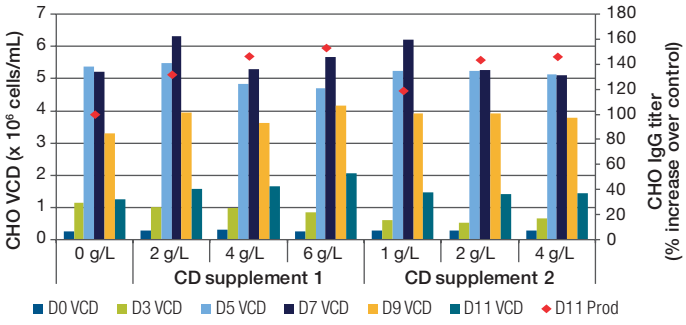

Figure 3. CD supplement titrations in a complete medium.

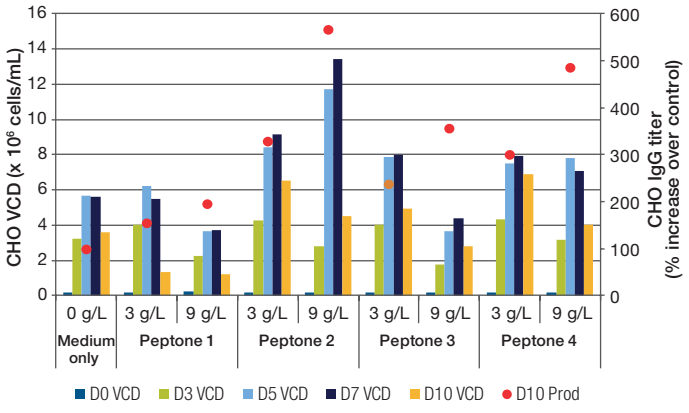

Figure 4. Peptone titration in a complete medium.

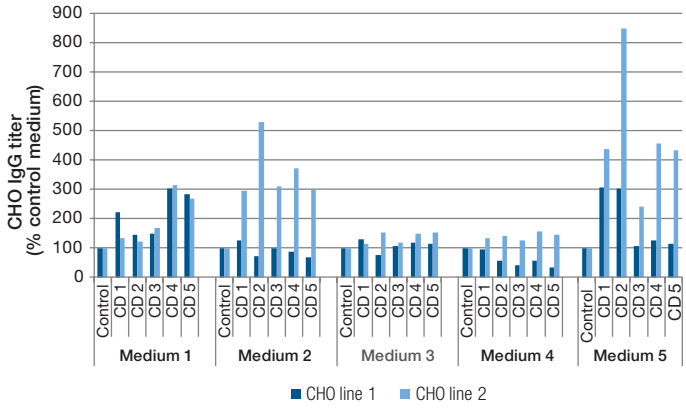

Figure 5. Performance comparison of multiple CD supplements in various media.

Once the appropriate supplements have been selected, create an experimental design in which each supplement is evaluated in the pre-selected base medium at a wide range of concentrations. Such a dose titration study will help identify the optimal effective concentrations of peptones (Figure 2) or CD supplements (Figure 3) for cell growth and protein titer. A cell line and base medium combination will respond differently to different peptones, as seen in Figure 4. In this study, four peptones were tested at two concentrations on a CHO cell line using the same base medium. Both concentration-dependent and peptone-specific effects were seen on cell growth and protein titer. Supplement performance can also vary with cell line or base medium. Figure 5 shows the differential performance of five CD supplements on two cell lines in five different base media. Note the variation in protein titer. Cell line–dependent effects of peptones are shown in Figure 6. Four peptones were tested on three CHO cell lines in their respective base media. In general, peptones enhanced protein titer compared to no peptone addition (control), although at varying levels, and either increased or decreased cell growth. A similar evaluation of seven CD supplements on three CHO cell lines resulted in a range of performance (Figures 7–10). Again, each CHO cell line was cultured in its respective base medium in the absence (control) or presence of each CD supplement. In most cases, the CD supplements increased protein titer above control levels, while either enhancing or suppressing cell growth.

Blends of peptones or CD supplements should also be considered since synergistic effects have been observed in some processes when multiple supplements were used [11]. A CHO line was evaluated with a blend of peptones, individual peptones, and without any peptone (Figure 8). For this cell line and base medium combination, peptone blends resulted in a significantly higher antibody titer compared to the other two conditions.

Significantly higher protein titers can be achieved with the identification of a supplement that meets the specific requirements of the cell line. Selection of the supplement should be based upon cell proliferation, protein titer, and protein quality data, since these parameters do not always correlate in a production process.

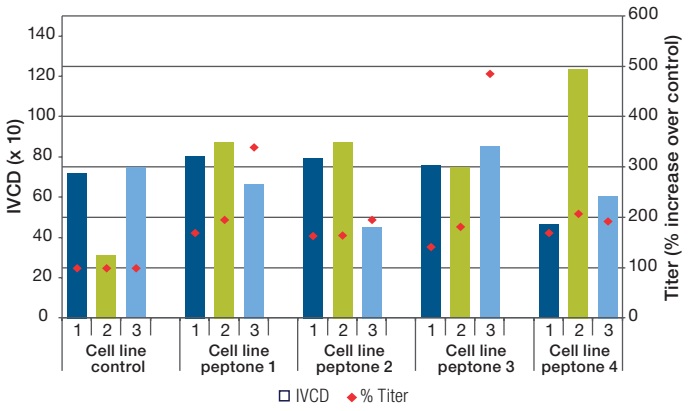

Figure 6. Performance comparison of CHO lines with multiple peptones. IVCD: integral viable cell density

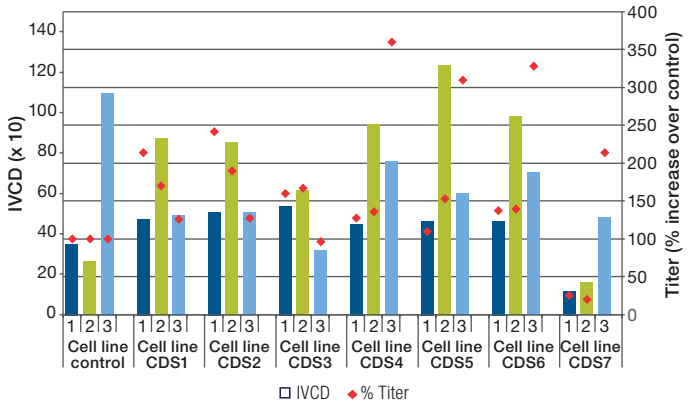

Figure 7. Performance comparison of CHO lines with multiple CD supplements.

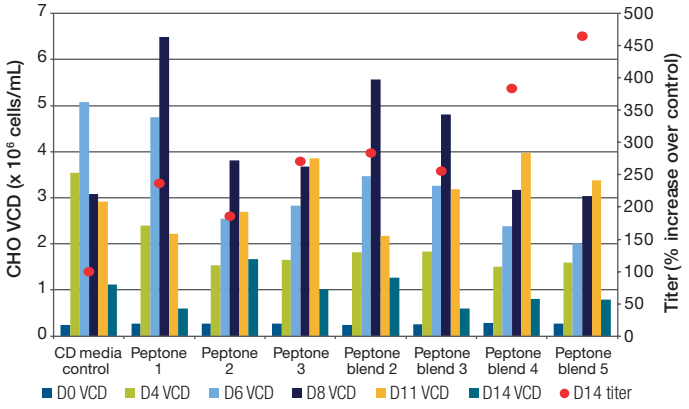

Figure 8. Performance comparison of individual and blended peptones.

Process optimization

The improved performance obtained through the identification of a new base medium and supplementation/feed can be further enhanced when coupled with effective process optimization [12]. Using spent media analysis to understand the cell's nutritional requirements makes it possible to design a feed strategy that greatly enhances the process performance. In some cases, a CD feed can be used. However, in many situations, a peptone-based feed can result in substantial increases in cell proliferation and protein titer. Feeding with a peptone can enhance cell growth and titer over batch culture (Figure 9A and 9B). In this case, peptone feeding was used to provide a rapid, simple solution to enhance cell performance without the extensive investment of time and resources to perform spent medium analysis and design a more complex feed solution. Similarly, CD supplements, when used as feeds, can also have a beneficial impact on cell cultures. Figure 10 illustrates the ability of a CD feed to enhance cell growth and protein titer.

Determining the appropriate supplementation and feed strategy is essential to achieving increased performance. Some processes require that the supplement or feed be present from the beginning of the run. Others perform best when the supplement or feed is added later in the process. Figure 11 shows the cell growth and protein titer results for four different feed strategies (1–4), where the same amount of total feed (10 g/L) was used but was delivered at varying time points and concentrations across the culture period. Based on the timing and concentration of feed addition, differing effects were seen in the cell growth profiles and the protein titer. Feed strategies 3 and 4 quickly produced high cell growth and protein titers. Feed strategies 1 and 2 promoted cell growth at more moderate levels, but extended culture longevity and achieved similar protein titers.

If paired correctly with the appropriate base medium for the cell line in use, peptones and CD supplements can enhance protein titer and deliver the desired level of protein quality. Appropriate protein quality is critical for the safety and efficacy of a mAb therapeutic.

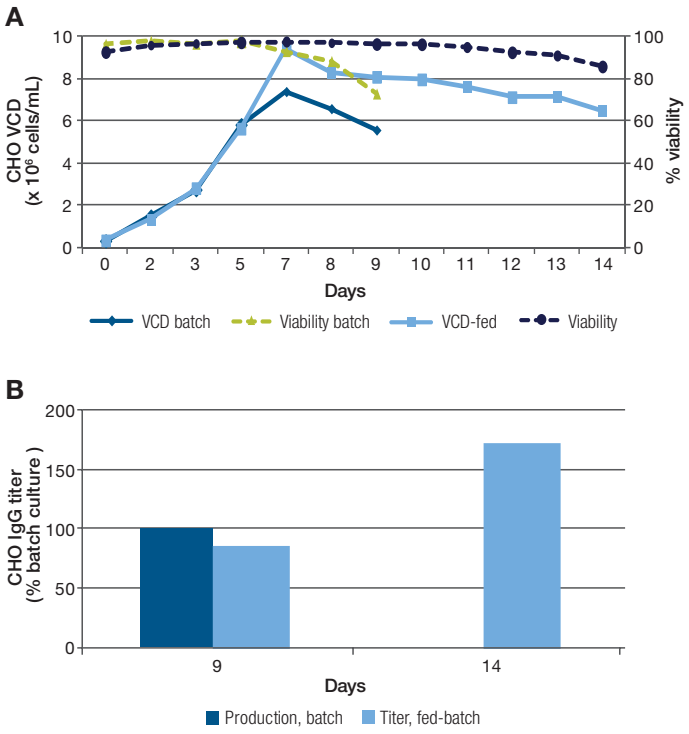

Figure 9. Evaluation of peptones used as bioreactor feeds.

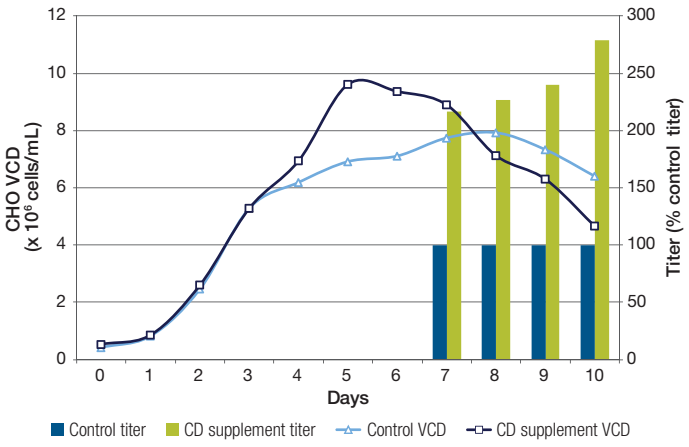

Figure 10. Evaluation of a CD supplement used as bioreactor feed.

Two primary quality attributes are the glycosylation and charge variant profiles. All antibodies are glycosylated at conserved positions in their constant region, which is critical for proper conformation and function. As shown in Figure 12, the glycosylation profile for a mAb can be modulated by peptone or CD supplementation.

There are multiple chemical degradation pathways that can lead to the formation of mAb acidic or basic charge variants. Controlling the levels of charge variant generation is key to the successful production of an effective therapeutic. The desired profile can be achieved through the utilization of either peptone or CD supplements. Figures 13 and 14 show how the desired mAb charge variant profile was achieved through the use of peptone or CD supplementation. The original medium formulation was designed with a peptone supplement, which gave an undesirable charge variant profile. The charge variant profile was corrected through the use of alternative peptone supplements, which were included in two separate media formulations (peptone media 2 and 3). Similarly, CD supplementation resulted in two of three media formulations (CD media 1–3) which produced the desired mAb charge variant profile. In both cases, protein titer was increased compared to the original medium (data not shown).

Having a thorough understanding of the production process is critical for maintaining consistent results. There are many potential sources of variability, and each source needs to be identified and controlled. Sources of variability include different manufacturers or lots of base media, the timing of addition and amount of key components added to the culture, potential sources of trace component contamination, and the use of generic manufacturing processes that are suboptimal for a particular cell line.

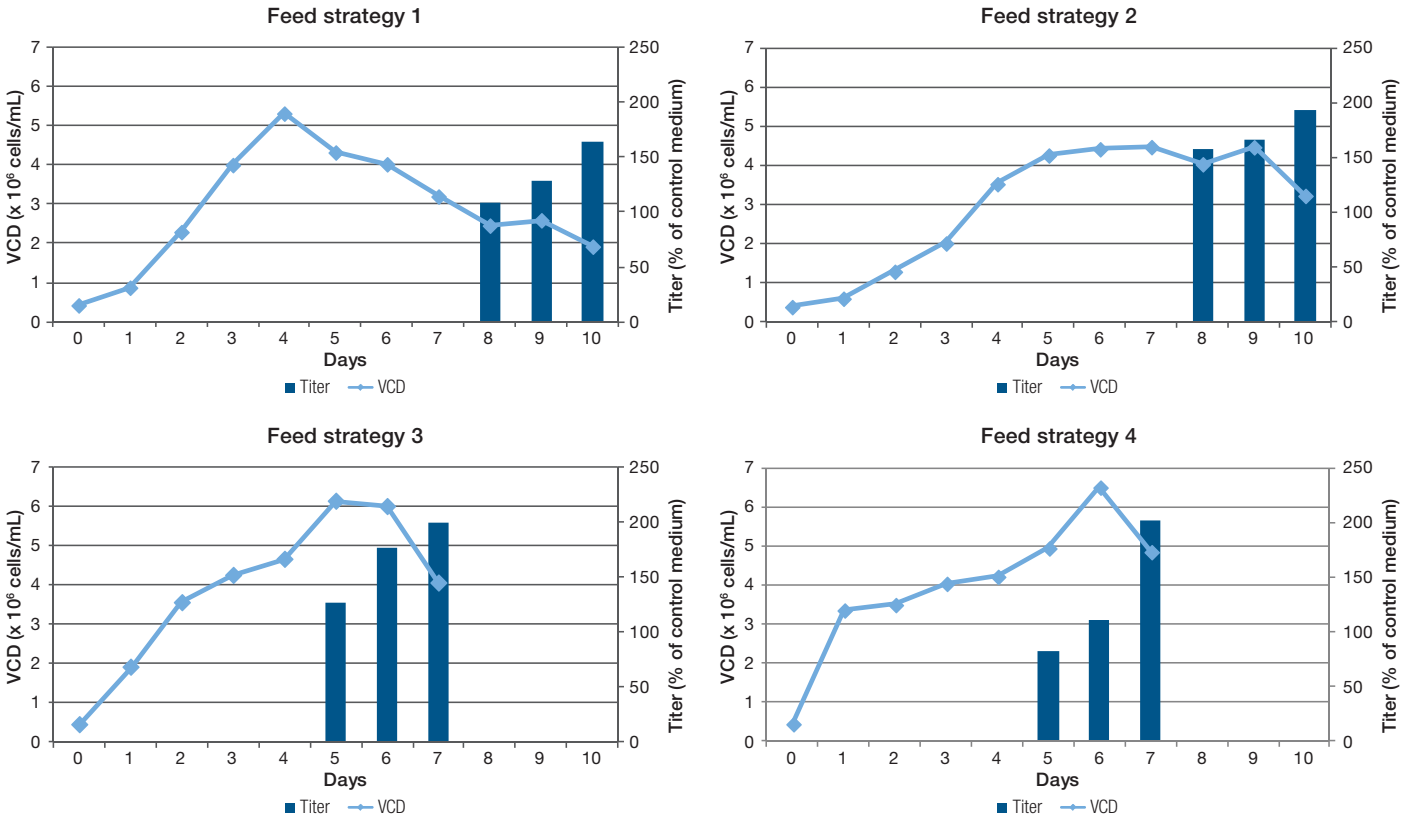

Figure 11. Feed strategy 1–4. Feed strategy 1 delivered 2.5 g/L on each of four days, including day 0. Feed strategy 2 delivered 5g/L on each of two days, including day 0. Feed strategy 3 delivered 2.5 g/L on each of four days. All days were distinct from feed strategy 1, except day 0. Feed strategy 4 delivered 5g/L on each of two days with no feed added on day 0.

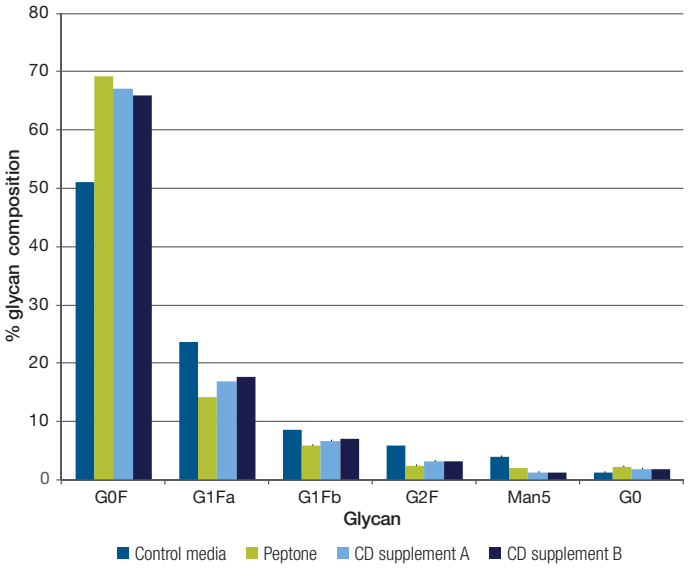

Figure 12. Glycan analysis.

Peptones and CD supplements designed for the biopharmaceutical industry are manufactured and released to strict specifications. CD supplements are advantageous due to their defined nature, but it is important to understand that trace contaminants inherent to CD formulations can affect process performance. Variability that is observed with peptones can be controlled by characterizing the process to understand the critical factors contributed by the peptone. Through a comprehensive analysis of the spent media from multiple production runs using multiple peptone lots, key component concentrations can be identified and maintained at the appropriate levels. Once the process is properly controlled, the expected results will be consistently achieved. An optimized upstream process translates to a quality downstream product.

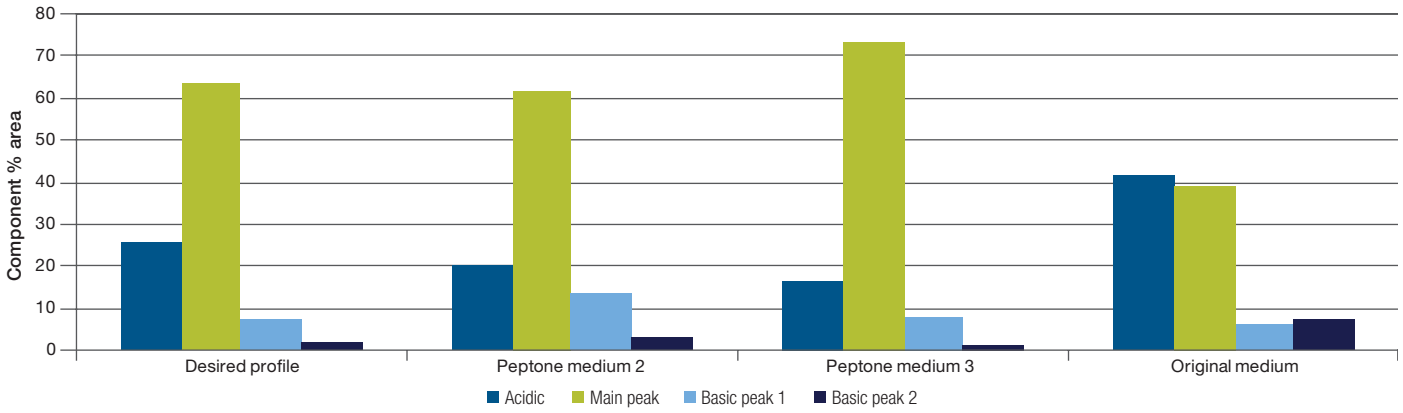

Figure 13. Charge variant profile in peptone-containing media.

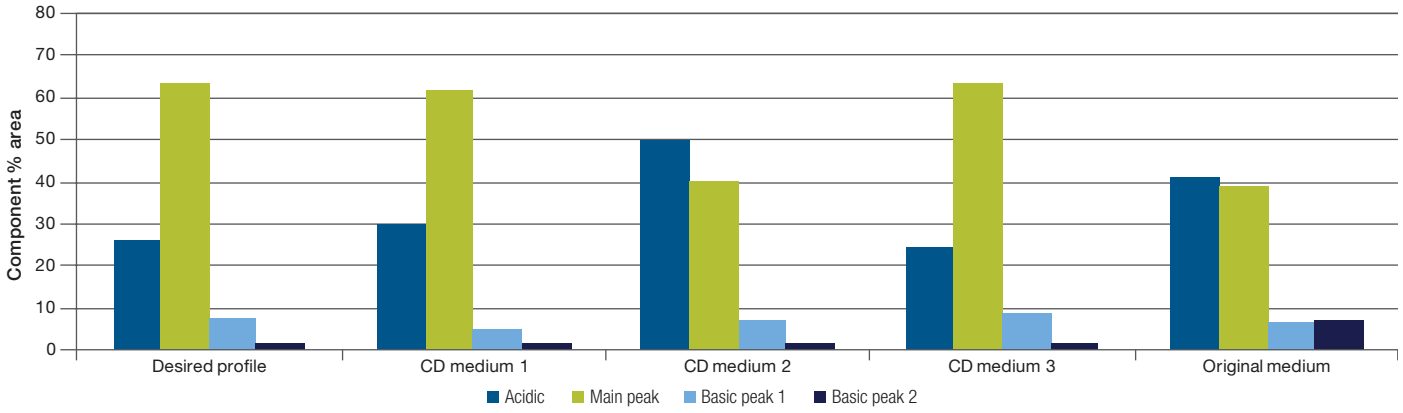

Figure 14. Charge variant profile in CD supplement–containing media.

Media Design Service

It would be ideal to use a single medium across multiple cell lines and projects. However, to meet performance goals, diverse cell requirements often demand the optimization of media and supplements for individual cell lines. Since this work takes significant time and resources, one may decide to eliminate many potentially critical design points, and thus risk missing production or quality goals. To address this need and ensure the identification of the optimal medium formulation that meets production and product quality goals, we offer a Media Design Service (MDS). Our team of dedicated, experienced scientists will work with you in a highly collaborative process to develop a medium and/or feed formulation that meets your performance requirements. We offer a library of diverse chemically defined (CD) media as well as a distinct and diverse peptone-supplemented media library. These libraries, coupled with our CD and peptone supplements and feeds, are designed specifically for the biopharmaceutical industry. Proprietary design of experiments (DOEs) are used to optimize a base medium specifically for the cell line, or rapidly identify the appropriate supplementation. We can use our vast analytical capability to perform spent media analysis quickly. Through MDS, we partner with you from initial screens through final scale to ensure an optimized process at each step.

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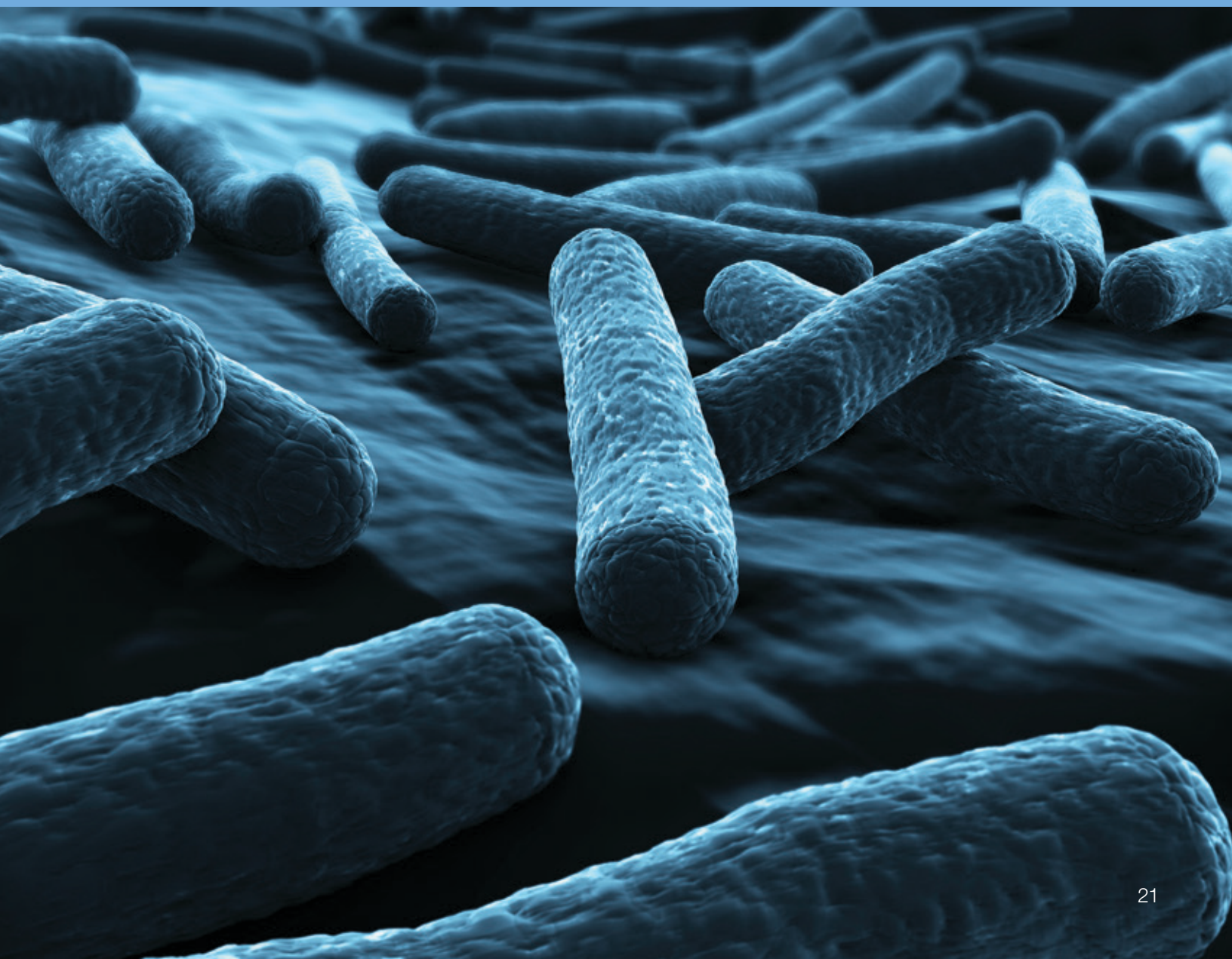
Fermentation applications

Fermentation medium will generally consist of a nitrogen source, such as amino acids, carbon source, such as sugars, phosphates for buffering, trace metals, cofactors, vitamins, and other essential components. The composition of the fermentation medium will vary greatly as per the nutritional requirements of the microorganism of interest and other factors in the media, which affect optimal drug production. Such multifaceted nutritional needs for fermentation applications can be met using peptone-containing media, also known as complex media.



Peptone-containing media have a long and proven history for production of bacterial vaccines, such as diphtheria, tetanus toxoid, and *Haemophilus*. Additionally, peptone-containing media have been extensively used for recombinant protein production using bacterial hosts, such as *Escherichia coli* and yeast hosts like *Pichia pastoris* [1–4].

Peptone-containing media have several advantages as they are relatively inexpensive, they support a wide variety of growth from a large group of microorganisms, they promote the growth of the more fastidious organisms, they stimulate toxin production, and they routinely produce higher yields.

Fermentation media design

The role of the medium is to provide essential nutrients that can be utilized and integrated into the dividing cells of the fermentation. Selection of medium components can have an impact on the growth, function, and even the genetic stability of cells, *in vitro*. A properly designed fermentation medium should contain a carbon source, such as glucose, glycerol, or other fermentable sugar, balanced to a nitrogen source, such as ammonium salt or amino acids to meet the metabolic needs of the microorganism for maximizing growth and production. Media components such as sodium and potassium salts and phosphates should be optimized to achieve the appropriate osmotic balance and desired pH. Additionally, essential growth factors (i.e., vitamins and trace minerals), could play a critical role in successful media design [5].

Peptones are a single source of many of these components and can be used at higher concentrations of up to 30 g/L for fermentation media. When used in fermentation media, peptones provide a major source of both carbon (via their carbohydrate content) and nitrogen (via amino acids and peptides.) Additionally, peptones provide buffering salts, such as phosphates, or essential salts of sodium, potassium, calcium, or magnesium, as well as essential micronutrients like trace metals and vitamins. The amino nitrogen (AN) content and the AN/TN ratio (amino to total nitrogen) are important in understanding the overall carbon to nitrogen ratio in the peptone and the final medium when designing a medium for bacterial systems.

Typically, it is simpler to design a medium for rapid initial growth than for maximum product accumulation, especially in the case of secondary metabolites. It is essential to understand the dynamics of your organism's product production. Phosphate levels and the presence/absence of iron are often cited as affecting secondary metabolite production [6]. Calcium, zinc, copper, and nickel may be important ions in understanding secondary metabolite effects for toxin production during vaccine development [7, 8]. In addition, pH during fermentation is often cited as a critical parameter for both growth and secondary metabolite production in bacterial systems and can be influenced by peptone selection and media design [9]. Understanding the key media drivers for your organism and combining them with the requirements for the process is critical for a successful media design.

Selecting a peptone

Successful media development is a multifaceted process. In order to comprehensively cover all the variables with the least time and effort, it is usual to employ statistical methods [10]. When initially selecting candidate peptones, consider the typical analyses data for elemental concentrations, amino acid profile, the degree of hydrolysis, and carbohydrate content to match the nutritional needs of the microorganism. For example, certain anaerobic bacteria might prefer media that are richer in certain specific amino acids, for anaerobic fermentation processes [11]. Further considerations might be given to animal origin suitability based on the end application as well. As an example, for animal health vaccines (like clostridial vaccines), an animal origin peptone might be a suitable option since they have a long history of enhancing toxin production. For human health applications, such as those produced in recombinant *E. coli*, animal origin–free peptones, such as yeast extracts and soy peptones, can be a suitable option [12].

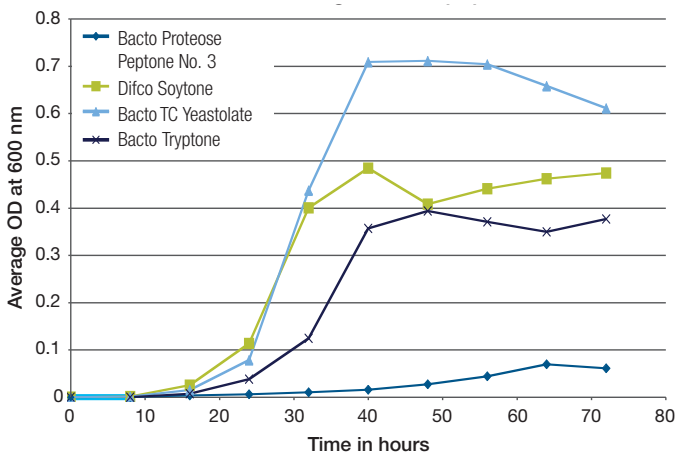


Figure 1. *S. cerevisiae* growth on peptones.

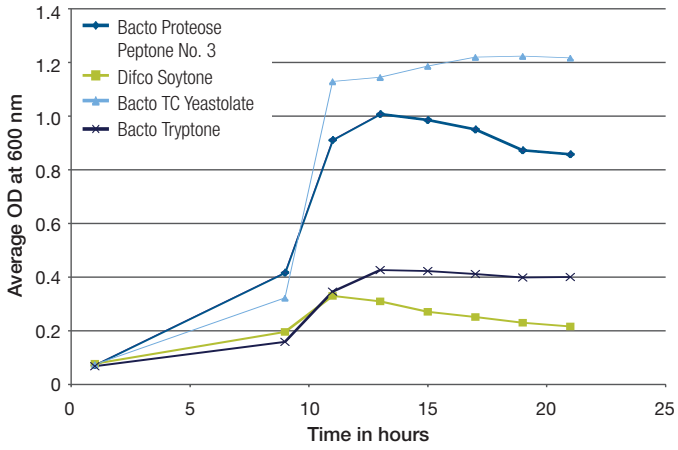


Figure 2. *E. faecalis* growth on different peptones.

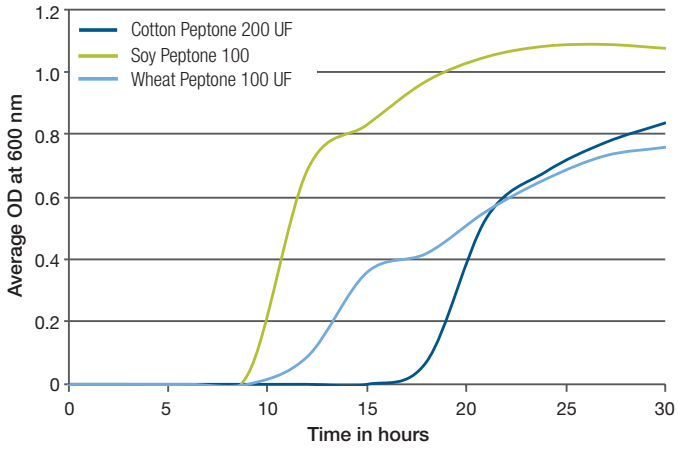


Figure 3. *E. coli* growth on different peptones.

After selection of appropriate peptone candidates, individual experimentation with a variety of peptones is suggested to select the optimum peptone or combination of peptones. As an example, Figures 1 and 2 depict the growth of *S. cerevisiae* and *E. faecalis* on various animal origin–free and animal origin peptones. As seen, each microorganism has different growth profiles for different peptones, highlighting the need to evaluate multiple peptones for optimal selection.

Moving to animal origin–free media components

A goal for the development of new fermentation products has been to either source TSE/BSE-relevant raw materials from an OIE Negligible Risk Country or reformulate the media using animal origin–free components [13]. Our team has led the introduction of animal origin–free peptone supplements such as yeast, soy, and more recently, other plant-based peptones derived from bases such as cotton and wheat.

Figure 3 shows the evaluation of different animal origin–free peptones for an *E. coli* strain, where good growth is seen.

Another example of enhanced performance is shown in Figure 4. *E. faecalis* was grown side-by-side in formulations containing animal origin peptones or animal origin–free components in bench-scale fermentors. In this case growth enhancement, as measured by the mass or OD reading, doubled when the medium formulation was changed to all animal origin–free components.

Figure 5 shows growth curves for two of the four different soy peptones available from our portfolio. It also demonstrates the differing responses an organism may have to different peptones made with the same starting materials. For an *E. coli* with a plasmid, Gibco™ Difco™ Soytone provides better growth support than Gibco™ Difco™ Tryptone. In this experiment, the peptones were in 2% solutions with some buffering salts. The purpose of the experiment was to observe the growth performance with each peptone.

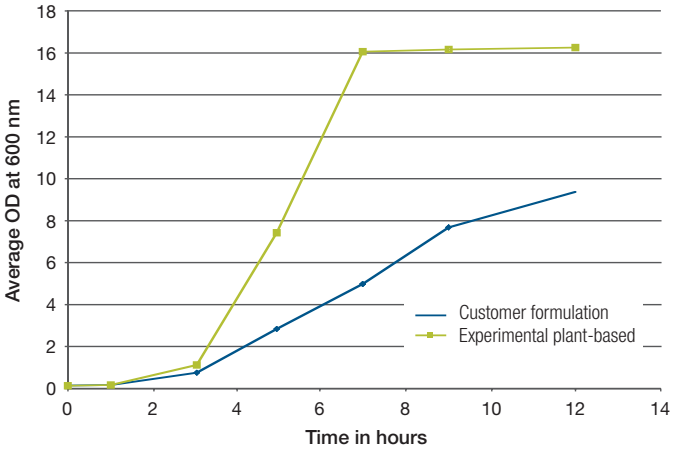


Figure 4. Comparison of *E. faecalis* growth on traditional media vs. plant-based media.

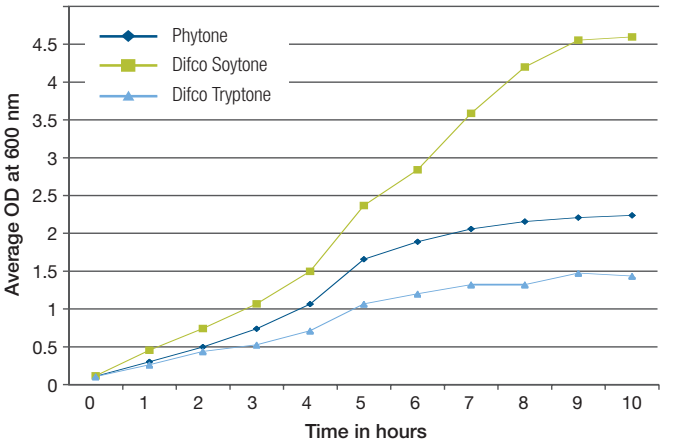


Figure 5. Growth of DH5α *E. coli* with plasmid on various peptones.

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Animal origin–free peptones

Historically, microbial and mammalian cell lines have been propagated in media containing animal components, including serum. In recent years, media formulators have been moving to eliminate animal-sourced components in media formulations, motivated by BSE/TSE risks in the 1990s [1]. In response, our team has developed a broad offering of yeast extracts, soy peptones, and other animal origin–free media and supplements.

Peptones derived from animal origin–free sources provide unique nutritional profiles suitable for both bacterial fermentation and mammalian cell culture applications. Yeast extracts and yeastolates offer a different range of nutritional choices to enhance production. Soy peptone, one of the first successful plant-based peptones to be optimized into cell culture, is processed in several different ways to provide various nutrient mixes to meet different needs. In addition to these more common animal origin–free components, many other plant-based products have been developed, including malt-, cotton-, and wheat-sourced peptones, which offer an additional breadth of supplements with strong and varied performance characteristics.

Yeast extracts

Yeast extract is defined in The United States Pharmacopeia (USP) as "a water-soluble, peptone-like derivative of yeast cells (*Saccharomyces*)." A primary yeast, such as baker's yeast (*Saccharomyces cerevisiae*) is grown specifically for use as a substrate in a bioprocess or as a food product/flavoring, on a sugar-based medium optimized for the specific yeast [2]. Manufacture of baker's yeast is a reproducible and controlled process. Yeast extract is an autolysate; i.e., cell hydrolysis is performed by the endogenous enzymes of the organism. It is typically available as a spray-dried powder and has been long recognized as a major source of B-complex vitamins by the health food industry. In contrast to food applications, our yeast extracts are developed with specific focus on the biopharmaceutical and bioproduction industries, which involves acceptance criteria that is relevant and industry specific applications. Yeast extract, as a supplement in a media formulation, supplies not only vitamins but also amino acids, peptides, carbohydrates, and some micronutrients.

Temperature, pH, the addition of other enzymes, type of medium substrate for the growth of *Saccharomyces*, and duration of autolysis are all variables that create the large variety of yeast extracts available.

Plant-based peptones

Soy peptones are enzymatic digests of soy flour. Defatted soybeans are heated or toasted in a processing plant under controlled conditions, in part to eliminate heat-labile protease inhibitors. The resulting soy flour is the principle substrate in a soy peptone and is rich in high-quality protein, carbohydrates, calcium, and other micronutrients [3].

Other animal origin–free peptones providing unique and specific nutritional profiles include malt extracts, developed from malted barley grains and which provide a strong carbohydrate source. Wheat and cotton peptones are digested for enhanced nutritional profiles like peptide content or nucleosides. These animal origin–free media sources represent a wide range of performance enhancing supplementation to match a wide range of bioproduction processes and cell lines, and also represent many opportunities for new sources to be tested and developed for the market.

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Bacto Yeast Extract

Yeast Extract

Bacto Yeast Extract, Technical

Difco Yeast Extract Ultra-Filtered (UF)

Difco Yeast Extract Low-Dusting (LD)

Product description

Yeast extracts are concentrates of the water soluble portion of autolyzed *Saccharomyces cerevisiae* cells. Our yeast extracts are derived from primary grown baker's yeast. Yeast extract is used extensively for many animal origin–free formulations for bacterial, fungal, mammalian, and insect cell culture. Yeast extract provides essential water soluble vitamins, amino acids, peptides, and carbohydrates to any medium formulation.

Potential applications

Our yeast extracts are animal origin–free products suitable for use as multi-functional nutritional supplements in mammalian cell culture, microbial fermentation and insect cell culture applications.

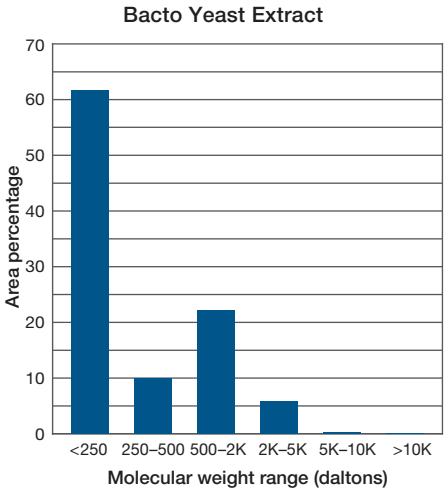
Bacto Yeast Extract is one of the most complete and versatile fermentation supplements available. It is an important ingredient for the microbiological assay of vitamins. Yeast extract is also of value in the assay of antibiotics. B factor, a growth substance necessary for the production of rifampin in a *Nocardia* species, can be isolated from yeast extract [1].

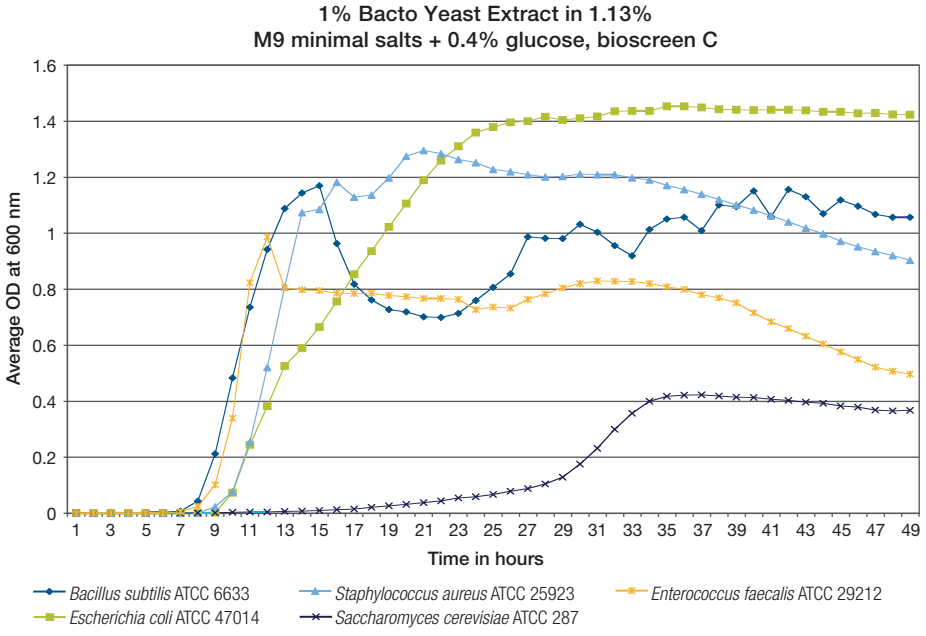
Gibco Yeast Extract was developed to provide a product for the biotechnology/pharmaceutical market with acceptable clarity and growth-promoting characteristics. Media formulations containing yeast extract are specified in standard methods for various applications [2-4].

Bacto Yeast Extract, **Technical**, was developed to provide products priced for the biotechnology market with acceptable clarity and growth-promoting characteristics.

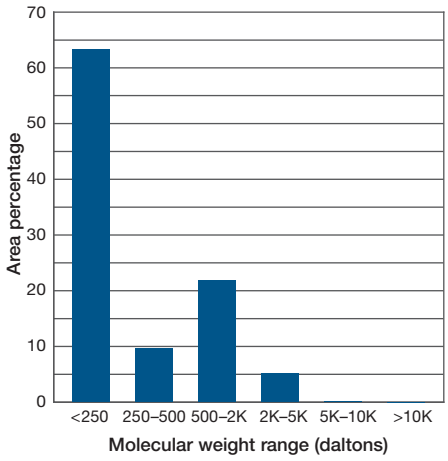
Difco Yeast Extract UF is ultra-filtered and specifically designed for mammalian cell culture applications. With its low endotoxin level and high content of naturally occurring B vitamins, it can be used effectively to reduce the need for fetal bovine serum. It has an endotoxin level of less than or equal to 500 EU/g.

Difco Yeast Extract Low-Dusting (LD) was designed to limit the amount of dust generated compared to other spray-dried yeast extracts.

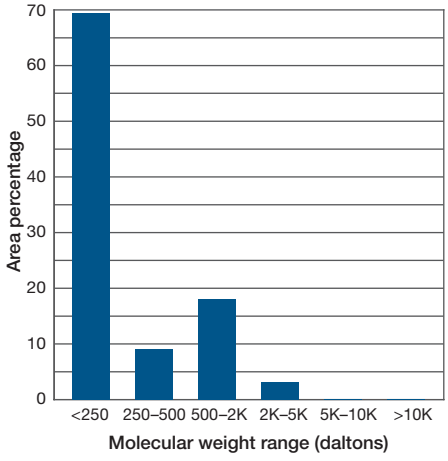




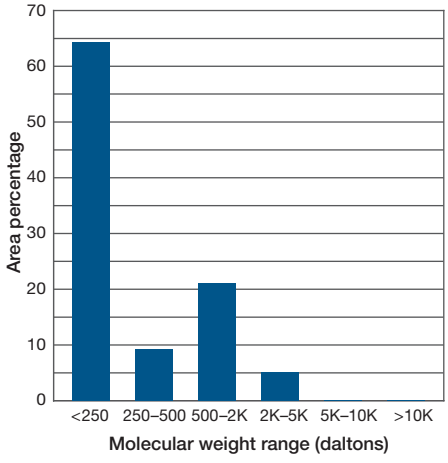
Difco Yeast Extract, UF



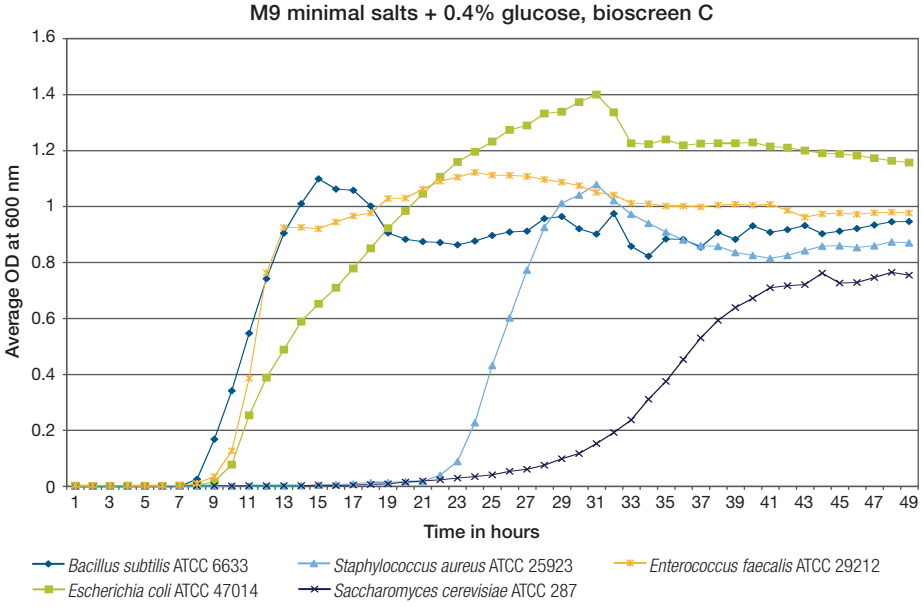
Gibco Yeast Extract



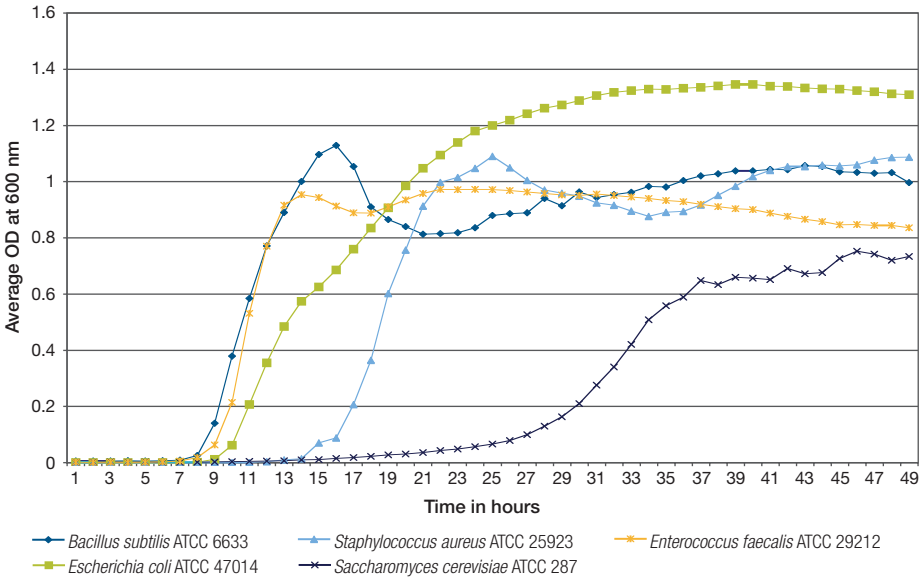
Bacto Yeast Extract, Technical



1% Difco Yeast Extract UF in 1.13%
M9 minimal salts + 0.4% glucose, bioscreen C



1% Yeast Extract 211929 in 1.13%
M9 minimal salts + 0.4% glucose, bioscreen C



Physical characteristics

Gibco yeast extracts are light to medium beige to medium tan, free-flowing, homogeneous powders containing up to a small amount of minute light to dark tan particles.

Ordering information

Product name	Size	Cat. No.
Bacto Yeast Extract	500 g	212750
	2 kg	212720
	10 kg	212730
	50 kg	212710
Gibco Yeast Extract	454 g	211929
	5 lb (2.3 kg)	211930
	25 lb (11.3 kg)	211931
Bacto Yeast Extract, Technical	500 g	288620
	10 kg	288610
Difco Yeast Extract, UF	500 g	210929
	10 kg	210934
Difco Yeast Extract, Low-Dusting	500 g	210933
	10 kg	210941

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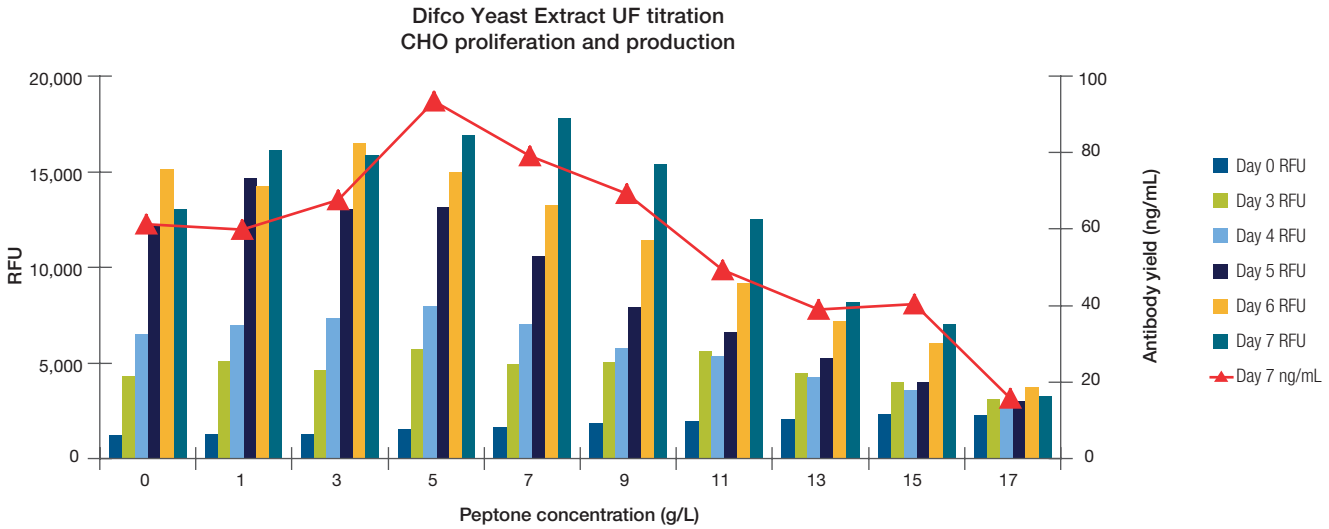


Bacto TC Yeastolate

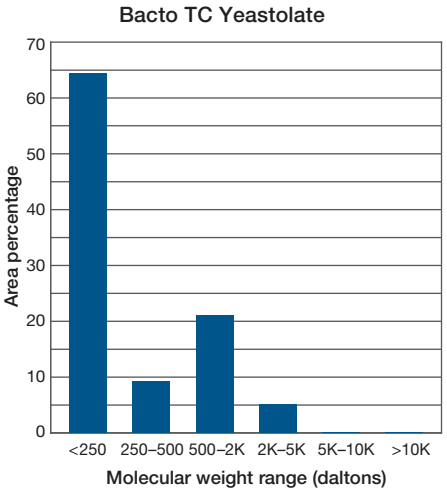
Difco TC Yeastolate Ultra-Filtered (UF)

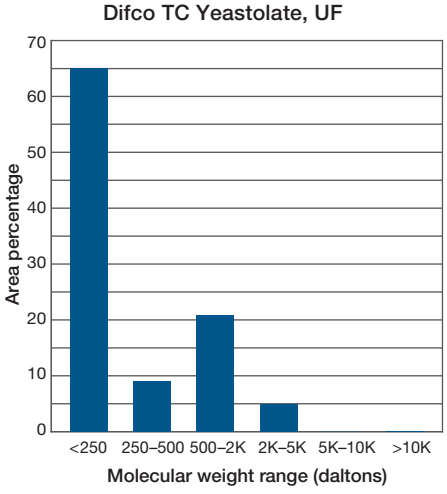
Product description

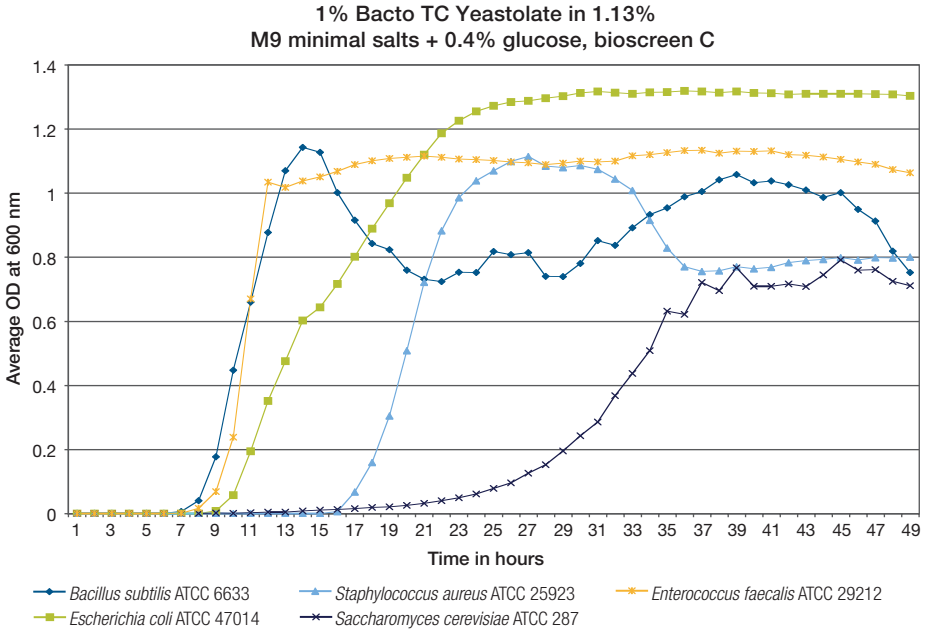
Bacto TC Yeastolate and **Difco TC Yeastolate UF** are animal origin–free and water-soluble portions of autolyzed yeast, enhanced for tissue culture (TC) applications. These products are a mixture of low molecular weight peptides, amino acids, carbohydrates (simple and complex), as well as vitamins. These TC yeastolate products meet extra requirements for reduced endotoxin, clarity, and suitability for the biopharmaceutical industry. Difco TC Yeastolate, UF has been ultra-filtered.

Potential applications

These products are intended as nutritional supplements for bacterial, insect, and mammalian cell culture. TC yeastolate has been used in insect cell culture. TC yeastolate was found to be a very versatile supplement to enhance growth and production characteristics of Sf9 and Gibco™ High Five™ cells [1-5]. Additionally, supplementation of TC yeastolate to CHO cell culture can enhance growth and mAb production.







Physical characteristics

Bacto TC Yeastolate is a beige-colored free-flowing, homogeneous fine powder.

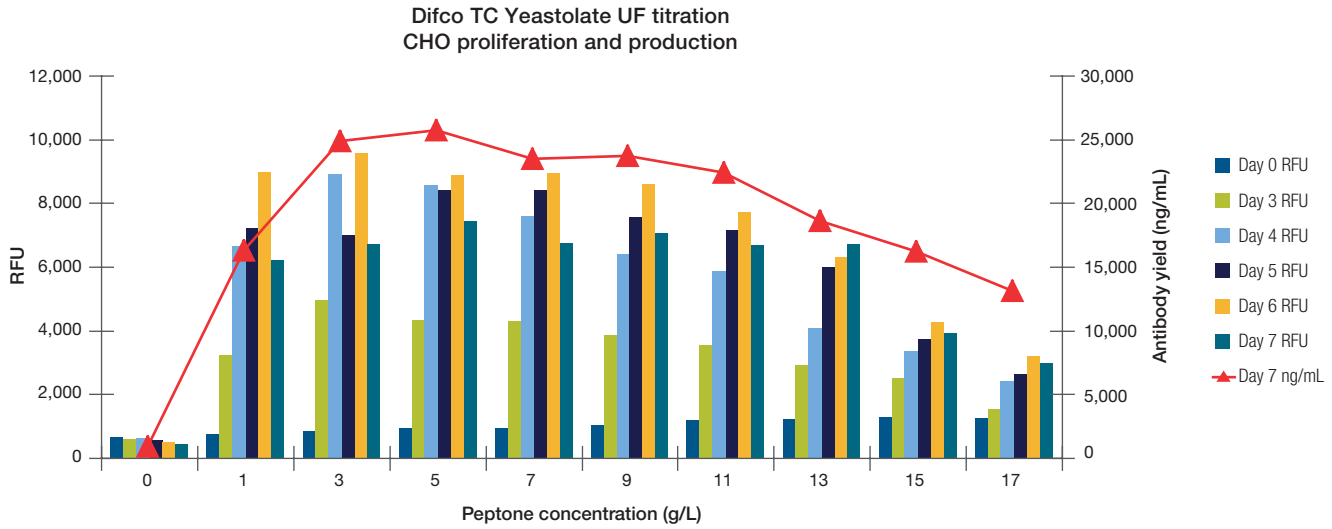
Difco TC Yeastolate UF is a beige-colored free-flowing, homogeneous fine powder.

Ordering information

Product name	Size	Cat. No.
Bacto TC Yeastolate	100 g	255772
	10 kg	255771
	25 kg	292731
Difco TC Yeastolate UF	500 g	292804
	10 kg	292805
	50 kg	670079

References

1. Chan, Greenfield and Reid. 1998. Optimising fed-batch production of recombinant proteins using the baculovirus expression vector system. *Biotechnol. Bioeng* 59:178-188.
2. Nguyen, Jarnagin, Williams, Chan and Barnett. 1993. Fed-batch culture of insect cells: a method to increase the yield of recombinant human nerve growth factor (rhNGF) in the baculovirus expression system. *J Biotechnol.* 31:205-217.
3. Ikonomou, Bastin, Schneider, Agathos. 2001. Design of efficient medium for insect cell growth and recombinant protein production. *In Vitro Cell Dev. Biol. Anim.* 37:549-559.
4. Bedard, Kamen, Tom, and Maassie. 1994. Maximization of recombinant protein yield in the insect cell/baculovirus system by one-time addition of nutrients to high-density batch cultures. *Cytotechnology* 15:129-138.
5. Donaldson and Shuler. 1998. Low-cost serum-free medium for the BTI-TN5B1-4 insect cell line. *Biotechnology Prog.* 14:573-579.



Bacto Malt Extract

Product description

Bacto Malt Extract is the water-soluble portion of malted barley. The extraction process breaks down the polysaccharides into simple sugars. After the malting process is complete, the extract is prepared from the malted barley by cracking the grain in a mill and then extracting the grain with a warm liquor. The resulting “wort” is filtered and evaporated or dried under vacuum [1,2].

Potential applications

Bacto Malt Extract is very high in carbohydrate content [3]. This product is suitable for the culture of yeasts and molds because of the high concentration of reduced sugars, especially the maltoses. Malt extract in the agar form is recommended for the detection and isolation of yeasts and molds from dairy products and foods and as a medium for stock culture maintenance.

Physical characteristics

Bacto Malt Extract is a light to medium tan, free-flowing, homogeneous powder.

Ordering information

Product name	Size	Cat. No.
Bacto Malt Extract	500 g	218630
	10 kg	218610

References

1. Bridson and Brecker. 1970. Design and formulation of microbial culture media. In Norris and Ribbons (ed.), *Methods in Microbiology*, vol. 3A. Academic Press, New York.
2. How malt is made, Briess Malting Company. 2 Dec. 2002. http://www.briess.com/HomebrewNew/hbhow.htm.
3. Cote. 1999. In Flickinger and Drew (ed.), Encyclopedia of Bioprocess Technology: Fermentation, Biocatalysis, and Bioseparation. John Wiley & Sons, Inc., New York.

Phytone Peptone

Difco Phytone Supplement Ultra-Filtered (UF)

Difco Soytone

Bacto Soytone

Soy Peptone 100

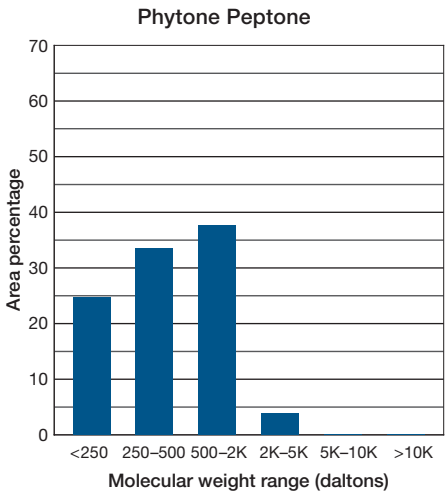
Product description

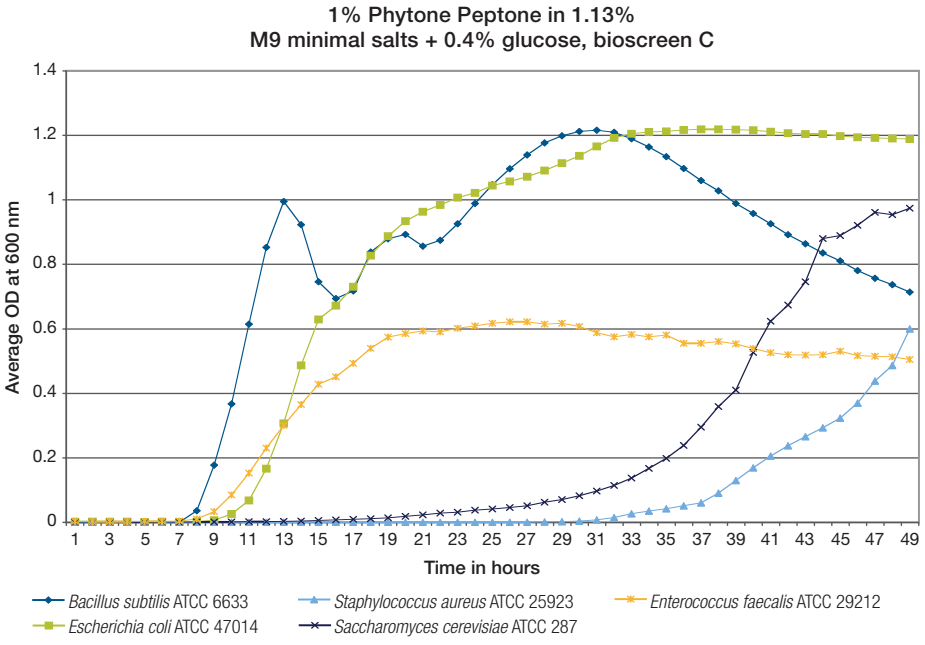
All of our soy peptones are enzymatic digests of soybean meal/flour. They are recommended for use in media for the cultivation of a wide variety of organisms, including fungi. These peptones contain naturally occurring high concentrations of carbohydrates.

Potential applications

We offer a diverse choice of soy peptones. The individual characteristics of each peptone are the result of processing methods engineered to consistently deliver these characteristics from batch to batch. The nutritional requirements of microorganisms and cell lines vary according to each individual strain. While some organisms or cell lines may prefer short chain or free amino acids, others benefit from longer chain amino acids. While the typical analysis profiles for each peptone in this manual can help direct the users to the correct peptone match, it is recommended that users supplement the typical analytical information with evaluations in their own individual growth models.

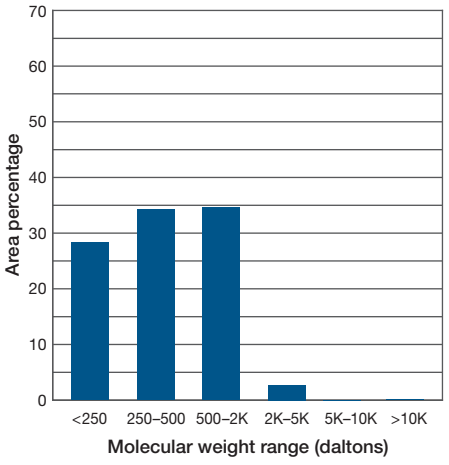
Phytone Peptone retains the high carbohydrate content of the soy plant tissue. It is an excellent peptone for the cultivation of fungi and fastidious types of bacteria, such as members of the *Clostridium* and *Neisseria* genera [1]. It has been used in mammalian cell culture applications.

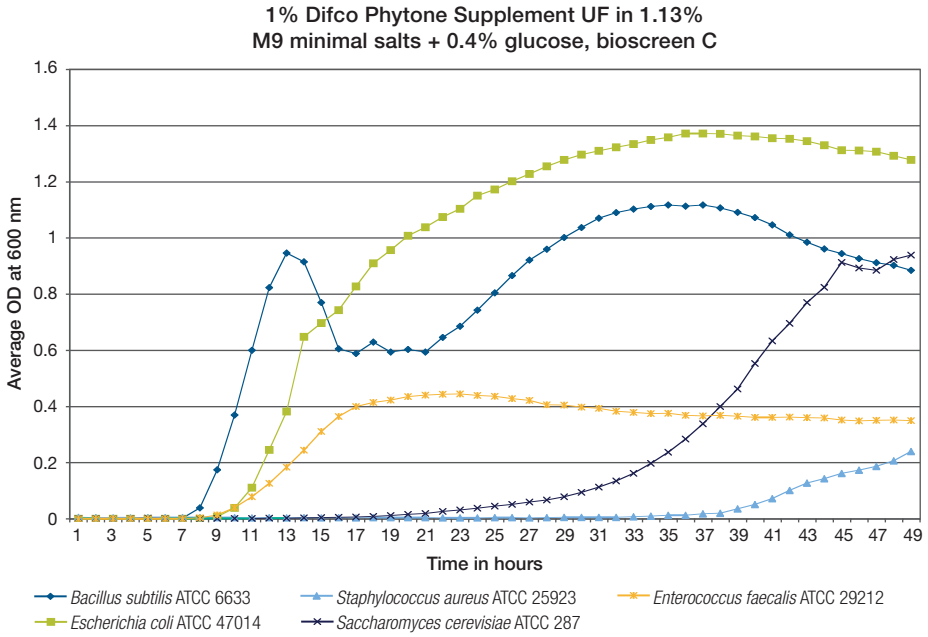


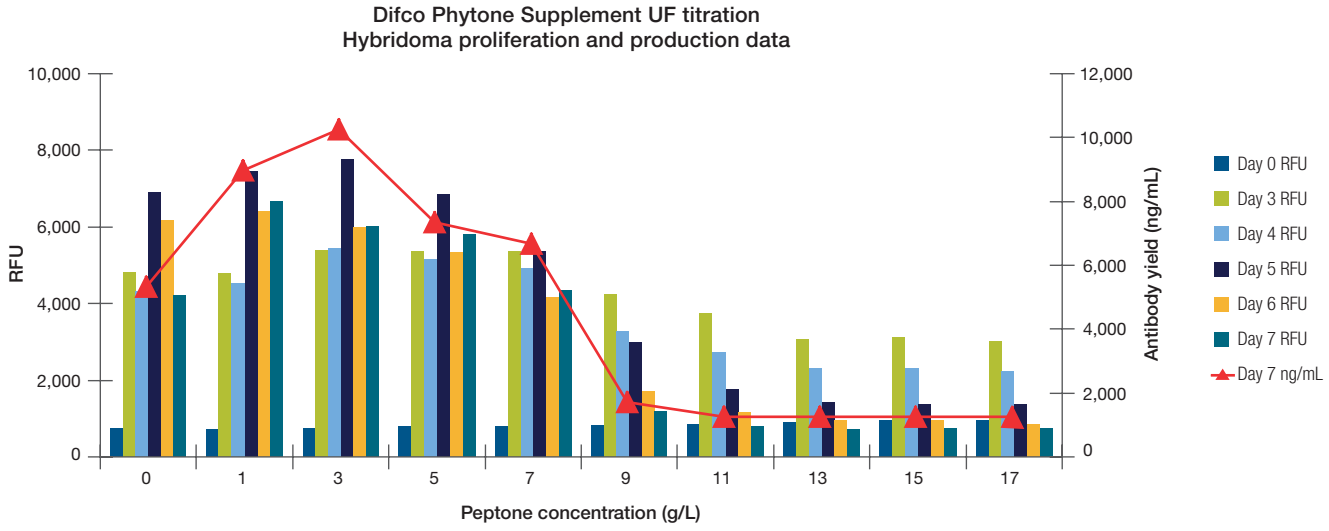


Difco Phytone Supplement UF is an ultra-filtered peptone developed specifically for the mammalian cell culture market. The nitrogen content of this product can be used to support growth and protein production in cultures where this component is a key factor. It has an endotoxin level of ≤500 EU/g.

Difco Soytone demonstrates excellent growth support for *Escherichia coli*. Subtle differences in the digestion process give Difco Soytone improved performance in cell culture.

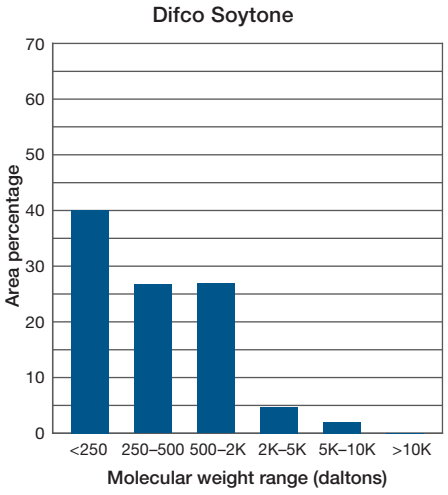


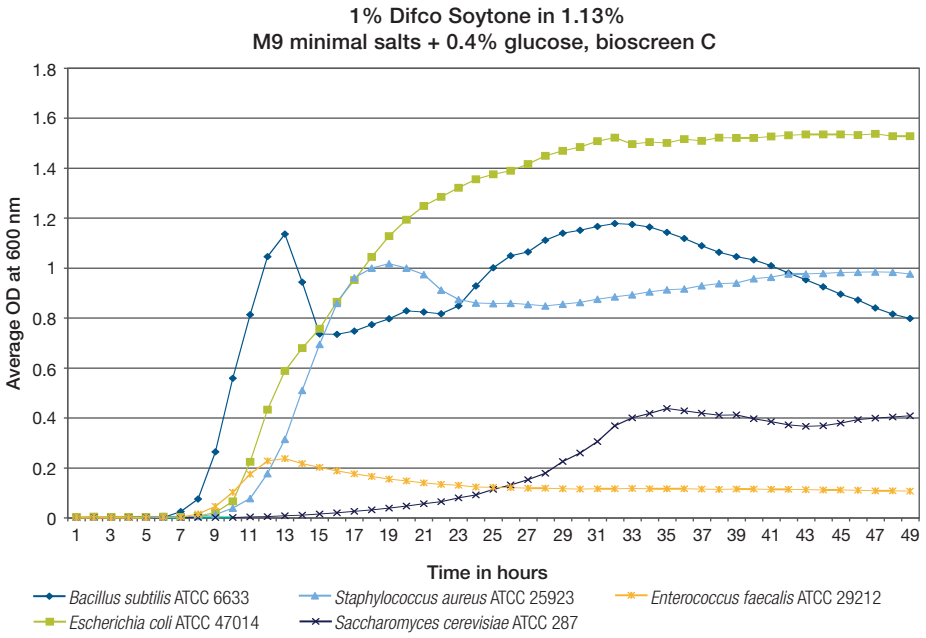


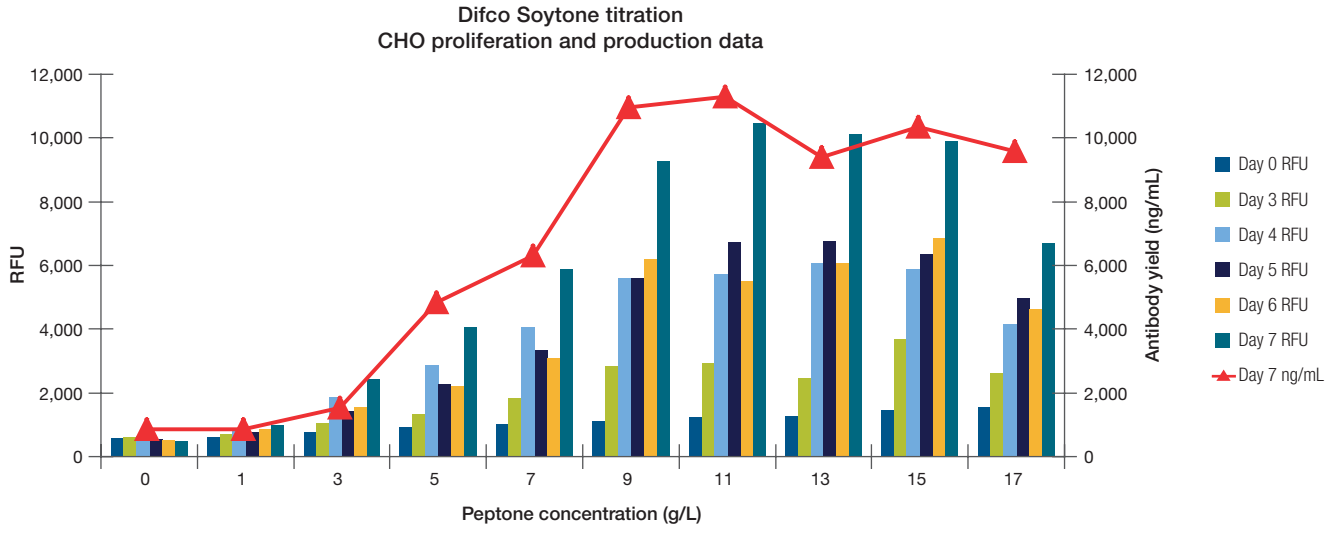


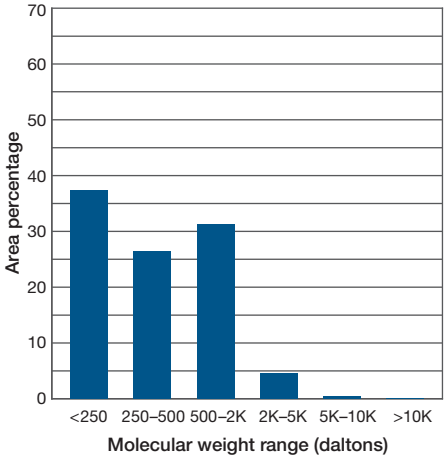
Bacto Soytone was found to be effective in the recovery of stressed *Escherichia coli* [2]. It was found that Bacto Soytone, with the addition of seven vitamins, replaced yeast extract as an economical alternative for the production of lactic acid by *Lactobacillus rhamnosus* [3]. Bacto Soytone utilizes an animal based enzyme in the digestion of the soy flour.

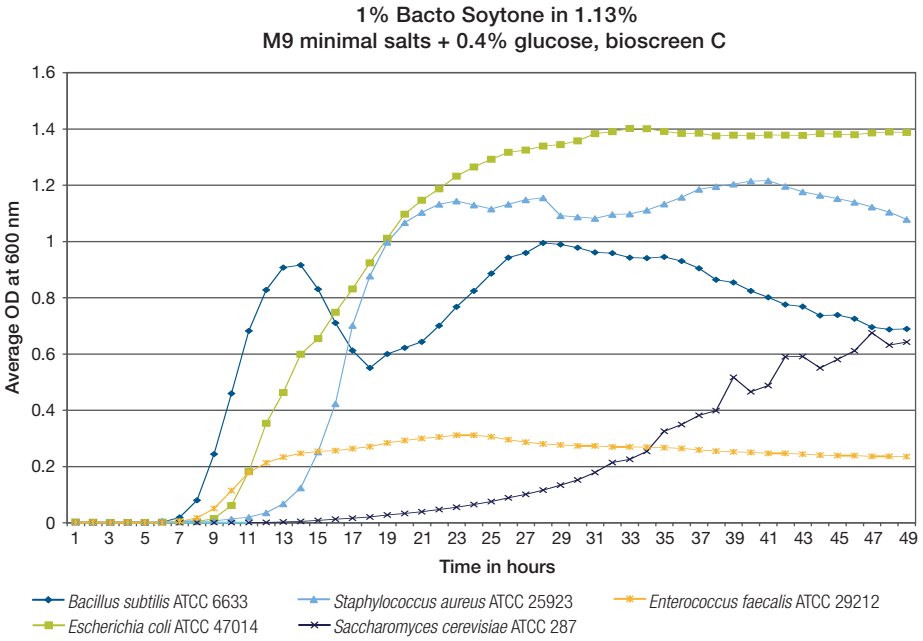
Soy Peptone 100 is a highly digested soy protein peptone rich in nucleosides and free amino acid content. It also has a high concentration of carbohydrates and vitamins, which is recommended for the cultivation of a wide variety of microbial organisms as well as varied mammalian cell culture applications. Soy Peptone 100 can be blended with other animal origin–free peptones, such as wheat or yeast, when looking for replacements for animal origin peptones.











Physical characteristics

Phytone Peptone is a fine homogeneous powder free of extraneous material.

Difco Phytone Supplement UF is a fine homogeneous powder free of extraneous material.

Difco Soytone is a fine homogeneous powder free of extraneous material.

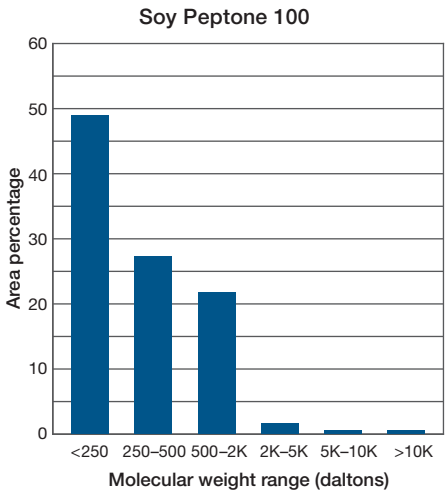
Bacto Soytone is a light to medium tan, free-flowing, homogeneous powder.

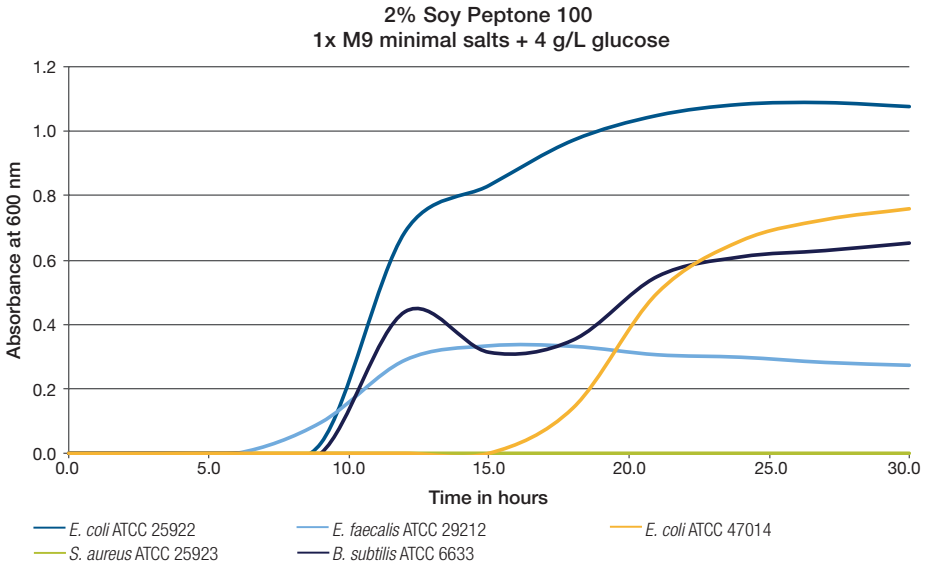
Soy Peptone 100 is a light to medium yellow to tan, fine, homogeneous powder, free of extraneous material. May contain a small amount of minute light to dark tan particles.

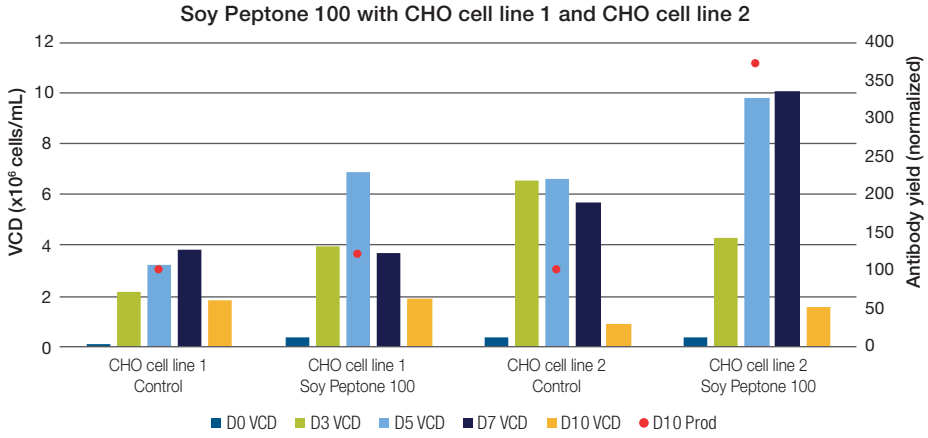
Ordering information

Product name	Size	Cat. No.
Phytone Peptone	454 g	211906
	5 lb (2.3 kg)	298147
	10 kg	292450
Difco Phytone Supplement UF	500 g	210931
	10 kg	210936
Difco Soytone	500 g	212488
	10 kg	212489
Bacto Soytone*	500 g	243620
	10 kg	243610
Soy Peptone 100	500 g	670138
	10 kg	670137

* Utilizes an animal-based enzyme in the digestion







References

1. Power (ed.). 1988. Manual of BBL™ products and laboratory procedures, 6th ed. Becton Dickinson Microbiology Systems, Cockeysville, MD.
2. Chou and Cheng. 2000. Recovery of low-temperature stressed *E. coli* O157:H7 and its susceptibility to crystal violet, bile salt, sodium chloride and ethanol. *Int J Food Microbiol.* 61:127-136.
3. Kwon, Lee, Lee, Chang, Keun and Chang. 2000. Production of lactic acid by Lactobacillus rhamnosus with vitamin-supplemented soybean hydrolysate. *Enzyme Microb Technol.* 26:209-215.

Wheat Peptone 100 Ultra-Filtered (UF)

Product description

Wheat Peptone 100 UF is hydrolyzed wheat gluten protein that has been digested for enhanced peptide content, resulting in a low concentration of free amino acids and carbohydrates. It is an ultra-filtered supplement and is ideal for mammalian cell culture applications.

Potential applications

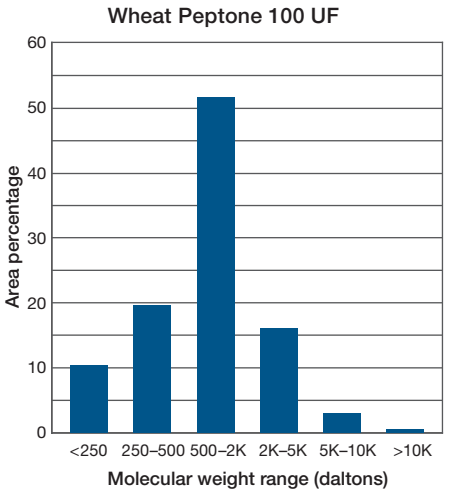
Wheat Peptone 100 UF contains glutamine that is associated with peptides, making it a stable source. This helps minimize the ammonia generation compared to the addition of free glutamine in cell culture media. Wheat Peptone 100 UF can also be used to enhance growth and production in microbial applications.

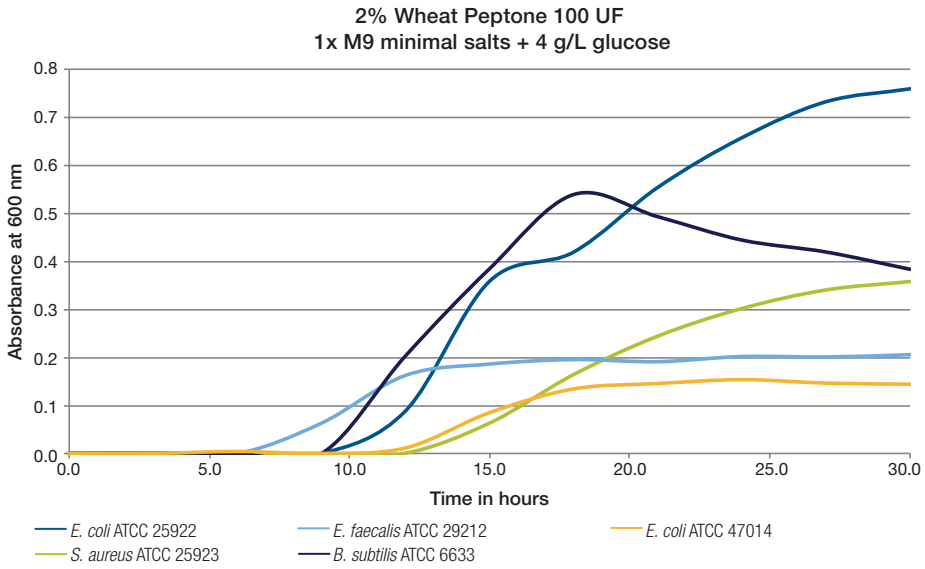
Physical characteristics

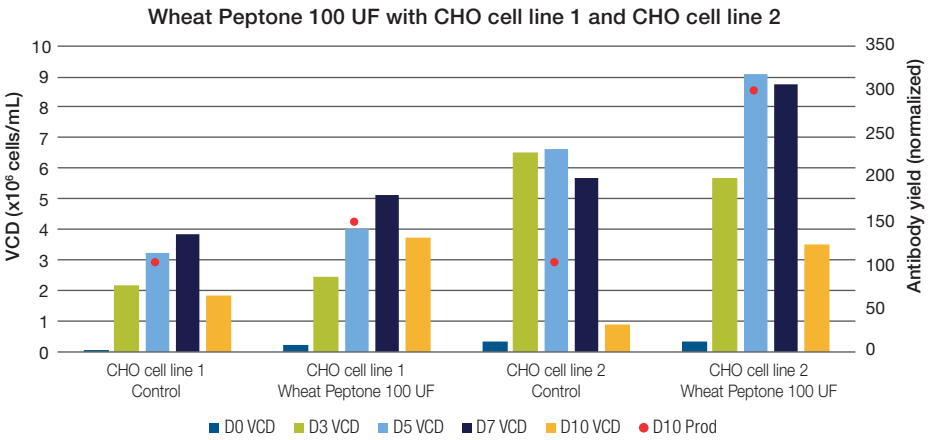
Wheat Peptone 100 UF is a light to medium yellow to tan, free-flowing, homogeneous fine powder. It may contain a small amount of minute light to dark tan particles.

Ordering information

Product name	Size	Cat. No.
Wheat Peptone 100 UF	500 g	670140
	10 kg	670139







Cotton Peptone 200 Ultra-Filtered (UF)

Product description

Cotton Peptone 200 UF is enzymatically digested cottonseed that has been ultra-filtered. This peptone is rich in nucleosides and carbohydrates and supports high level performance in production cultures.

Potential applications

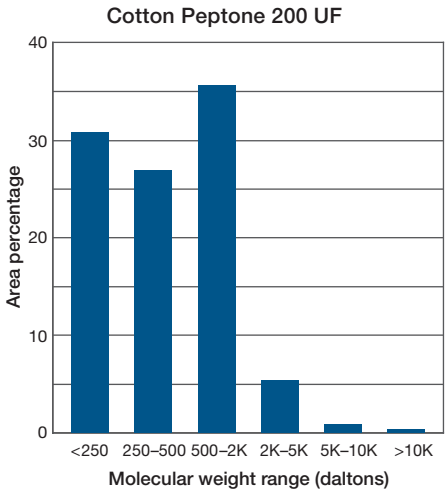
Cotton Peptone 200 UF has been optimized for use in bioproduction applications with either mammalian or microbial processes. This peptone is ideal as an alternate animal origin–free material source where yeast or soy products are not suited to the process.

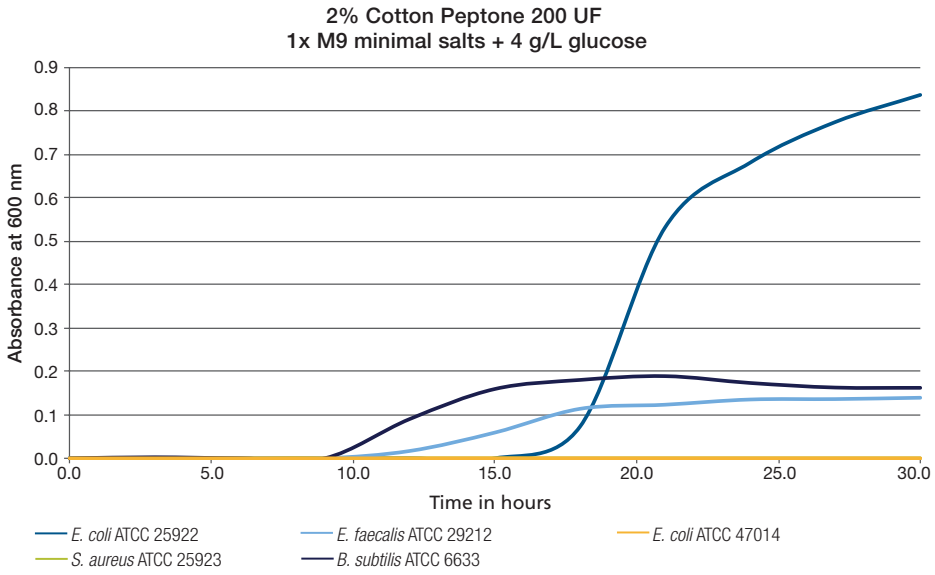
Physical characteristics

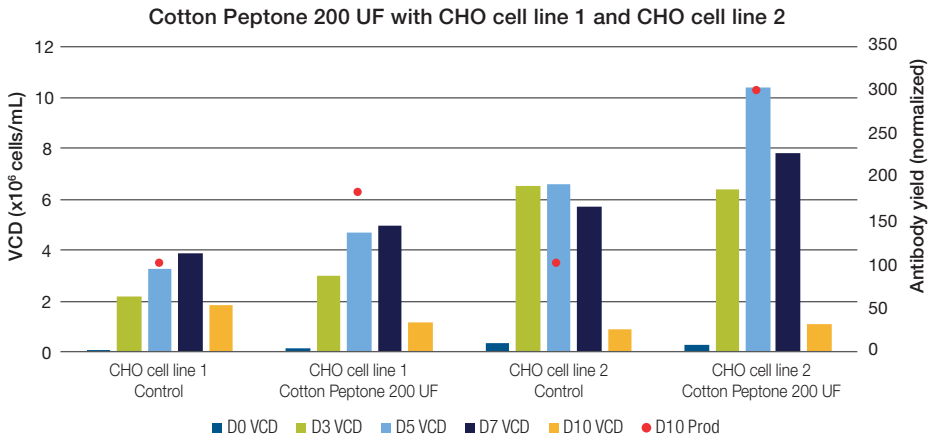
Cotton Peptone 200 UF is a light to medium yellow to tan, free-flowing, homogeneous fine powder. It may contain a small amount of minute light to dark tan particles.

Ordering information

Product name	Size	Cat. No.
Cotton Peptone 200 UF	500 g	670104
	5 kg	670105







Typical analyses: Animal origin–free peptones and yeast extracts

Gibco product	Total nitrogen (%)	Amino nitrogen (%)	AN/TN	Total carbohydrate (mg/g)	Ash (%)	Loss on drying (%)	NaCl (%)	pH (1% solution)	Calcium (µg/g)	Iron (µg/g)	Magnesium (µg/g)	Potassium (µg/g)	Sodium (µg/g)	Chloride (%)	Sulfate (%)	Phosphate (%)	Alanine (% free)	Arginine (% free)	Asparagine (% free)	Aspartic acid (% free)	Cystine (% free)	Glutamic acid (% free)
Bacto Malt Extract	0.3	0.3	0.97	995.4	0.3	3.1	0.2	5.2	111	5.8	130	603	713	0.07	0.07	0.08	0.1	0.0	0.0	0.0	0.0	0.0
Phytone Peptone	9.0	2.4	0.27	392.9	12.4	1.5	4.0	7.1	1001	89.8	2435	31547	34037	0.76	0.67	0.64	0.3	0.6	0.1	0.3	0.4	0.3
Difco Phytone Supplement UF	9.4	2.6	0.28	394.2	12.5	4.9	4.0	7.0	900	59.5	1700	21200	36100	0.76	0.58	0.71	0.3	0.8	0.2	0.2	0.5	0.4
Difco Soytone	9.2	3.7	0.40	336.2	10.7	3.5	0.0	7.0	250	61.0	1749	29787	31087	0.07	2.65	1.03	0.5	0.4	0.4	0.2	0.5	0.7
Bacto Soytone**	9.4	3.1	0.33	292.5	12.0	4.6	0.2	7.2	550	68.2	1610	22200	34040	0.17	2.33	0.82	0.4	2.1	0.3	0.2	0.4	0.4
Soy Peptone 100†	9	3.9	40	15.5	9.67	3.01	0	7.17	586	77.3	1282	32736	25596	0.22	0.30	0.42	1.1	2.3	0.7	0.5	0.3	1.5
Wheat Peptone 100 UF†	13.9	2.3	16	10.9	2.04	4.30	0	6.25	2600	3.1	391	3899	5182	0.65	0.04	0.17	0.1	0.0	0.0	0.0	0.0	0.3
Cotton Peptone 200 UF†	9.2	2.7	30	19.9	10.76	5.72	3	6.73	660	9.4	4046	22058	27927	4.34	0.57	1.41	0.3	0.6	0.3	0.4	0.0	0.5
Bacto TC Yeastolate	10.7	6.0	0.56	143.0	11.7	2.2	0.6	7.0	228	73.7	250	50850	8190	0.30	0.49	2.63	4.6	1.7	1.2	1.8	0.2	6.6
Difco TC Yeastolate UF	10.6	6.5	0.61	124.2	13.3	2.1	1.0	7.0	247	52.5	267	60940	3716	0.52	0.89	2.46	5.5	1.9	1.3	2.1	0.2	7.3
Bacto Yeast Extract	10.9	6.0	0.55	163.3	11.2	3.1	0.1	6.7	130	55.3	750	31950	4900	0.38	0.09	3.27	4.4	1.4	1.0	1.6	0.2	6.6
Gibco Yeast Extract	11.4	6.9	0.60	67.6	13.1	1.0	0.2	7.0	230	62.1	799	58013	1003	0.07	0.65	3.73	5.7	2.0	1.0	2.2	0.2	7.3
Bacto Yeast Extract, Technical	11.1	6.0	0.54	132.1	10.0	5.0	0.0	6.9	320	32.3	400	51030	760	0.12	0.55	1.10	3.3	2.5	1.4	1.5	0.9	5.7
Difco Yeast Extract, UF	10.7	6.0	0.56	108.2	18.2	0.7	0.0	7.0	191	57.9	558	59240	1244	0.13	1.02	2.70	4.8	1.5	1.2	1.7	0.2	6.8
Difco Yeast Extract Low-Dusting (LD)	11.45	5.9	0.52	8.2	11.1	4.0	0.0	7.0	263	40.0	272	54129	1202	0.11	0.69	1.72	3.8	1.5	1.5	2.0	0.0	5.9

Legend

☐ Free amino acids ☐ Total amino acids

0.0 Below limit of detection

The data in this table represent the typical amounts of each component in each product and are not specifications. Multiple lots of each product were tested and the results in the table are the averaged values of each component.

* Partially destroyed during hydrolysis

** Utilizes an animal based enzyme in the digestion of the soy flour

† The data in this table reflect the average results from multiple lots of non-GMP Preview material. These results are not representative of any specific lot of material.

	Glutamine (% free)	Glycine (% free)	Histidine (% free)	Isoleucine (% free)	Leucine (% free)	Lysine (% free)	Methionine (% free)	Phenylalanine (% free)	Proline (% free)	Serine (% free)	Threonine (% free)	Tryptophan (% free)	Tyrosine (% free)	Valine (% free)	Alanine (% total)	Arginine (% total)	Aspartic acid (% total)	Glutamic acid (% total)	Glycine (% total)	Histidine (% total)	Isoleucine (% total)	Leucine (% total)	Lysine (% total)	Methionine (% total)*	Phenylalanine (% total)	Proline (% total)	Serine (% total)*	Threonine (% total)	Tyrosine (% total)	Valine (% total)
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.2	0.1	0.0	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.0	0.0	0.1
	0.0	0.2	0.3	0.2	0.8	1.2	0.2	0.2	0.1	0.2	0.1	0.0	0.2	0.1	2.6	2.1	3.9	5.9	1.5	0.8	1.3	2.3	2.4	0.2	1.4	1.8	0.5	0.5	0.8	1.5
	0.0	0.2	0.1	0.2	0.9	1.5	0.2	0.3	0.1	0.3	0.1	0.1	0.3	0.1	3.1	2.4	4.7	6.5	1.8	0.9	1.6	2.7	2.8	0.3	1.6	1.9	0.6	0.6	1.0	1.7
	0.2	0.1	0.5	0.9	2.2	2.6	0.4	1.3	0.2	0.3	0.5	0.1	0.9	1.0	3.6	2.1	6.2	6.9	2.2	1.3	2.6	3.9	3.4	1.0	2.4	2.6	1.2	1.0	2.0	2.8
	0.1	0.2	0.2	0.6	1.7	1.9	0.3	1.2	0.2	0.3	0.2	0.2	1.3	0.4	2.5	2.8	5.5	8.9	2.1	1.1	2.8	4.3	2.9	0.5	3.1	2.0	1.4	1.1	1.3	2.7
	0.0	0.9	0.4	0.5	2.2	1.6	0.5	1.0	0.2	0.9	0.7	0.4	0.8	0.7	3.1	4.1	7.1	10.8	2.8	1.5	2.6	4.4	4.0	0.7	2.8	3.0	2.6	2.1	1.8	2.9
	0.2	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.2	1.9	2.2	2.2	30.0	2.9	1.5	2.4	5.2	1.0	1.0	4.2	10.3	3.6	1.7	2.4	2.8
	0.0	0.1	0.0	0.3	0.5	0.1	0.1	0.3	0.1	0.3	0.2	0.0	0.2	0.3	2.5	5.8	5.2	8.5	2.3	1.2	1.4	3.0	2.0	0.7	2.7	2.0	2.2	1.5	1.4	2.1
	0.3	1.3	0.5	2.1	3.5	2.3	0.8	2.3	0.9	1.5	1.3	0.6	0.8	2.4	4.6	2.4	4.8	8.7	2.7	1.1	3.6	4.9	4.2	0.8	3.3	1.8	1.4	1.4	0.9	3.7
	0.2	1.5	0.6	2.1	3.0	2.5	0.8	2.2	1.1	1.9	1.5	0.9	0.1	2.7	5.7	3.2	6.4	11.0	3.0	1.5	3.2	4.0	5.1	0.9	2.9	2.0	2.1	1.7	0.9	4.0
	0.2	1.0	0.4	1.8	3.0	1.9	0.6	2.0	0.8	1.3	1.1	0.5	0.8	2.2	5.6	2.6	5.3	9.4	3.0	1.3	3.0	4.1	4.6	0.8	2.6	2.0	1.6	1.6	1.2	3.5
	0.1	1.6	0.3	2.5	4.0	2.7	0.9	2.7	1.3	1.3	1.7	0.7	0.9	3.0	6.2	3.0	5.9	11.0	3.3	1.4	4.7	6.2	4.9	1.1	4.4	2.3	1.9	1.8	1.2	4.8
	0.6	1.1	0.6	1.9	3.5	2.0	1.1	2.1	0.8	1.9	1.0	2.2	0.8	2.5	3.6	3.4	4.8	8.5	2.2	1.0	2.3	3.7	3.8	1.0	2.2	1.7	2.3	1.8	1.0	3.0
	0.3	1.3	0.6	1.8	2.8	2.2	0.7	2.1	0.9	1.6	1.3	0.5	0.5	2.4	5.4	2.6	5.4	10.0	2.9	1.2	3.8	4.7	4.6	0.8	3.6	1.9	1.7	1.6	0.8	4.1
	0.3	1.1	0.5	2.1	3.7	1.9	0.8	2.1	1.0	2.0	1.6	0.6	0.5	2.5	4.8	3.1	6.4	10.7	3.0	1.3	3.3	4.7	5.0	1.0	2.7	2.4	2.8	2.8	1.1	3.8

Animal origin peptones

Despite the growing interest in animal origin–free peptone supplements, peptones from animal proteins remain effective and reliable for many cell culture and bioprocessing applications. Animal origin (AO) peptones are used in reducing or eliminating serum needs in certain media formulations and promote strong cell growth and toxin titers for vaccine production. AO peptones are derived from reliable and highly regulated raw materials. They provide a high-performing, cost-effective or supplement for processes and applications with less rigorous AO requirements.

Tissue-based peptones

Tissue-based peptones are proteins from animal sources that have been hydrolyzed into amino acids and peptides. Sources of animal protein for peptones can include various tissues, like muscle or pancreas; these tissues are derived from animals highly regulated for human consumption. A variety of proteases, such as pepsin and trypsin, may be used to accomplish enzymatic hydrolysis of animal protein. Tissue-based peptones can be tailored to specific nutritive needs by controlling the quality and origin of the protein, the quality and source of the enzyme used to digest the protein, and the methods used for hydrolysis, concentration, and drying the peptone.

Milk-based peptones

Milk-based peptones are derived from bovine milk, which is sourced under the same conditions as milk produced for human consumption. Milk is a complex material, consisting of water, lactose, lipids, salts, and proteins. Casein (80%) and whey (20%) are the primary protein components in milk.

Casein is one of the most nutritive of the milk proteins, containing all of the common amino acids and rich in the essential ones. Casein is precipitated with acid and then solubilized by addition of base. Casein peptones are then manufactured by acid or enzymatic hydrolysis of the solubilized material, which typically consists of 87% to 90% protein [1].

Whey, also called milk plasma, is the soluble material remaining after casein precipitation. Whey protein concentrates include the lactalbumin and lactoglobulin proteins and are recovered using separation technologies such as ion exchange and filtration. Lactalbumin recovery involves heat denaturing before separation [2].

Whey proteins undergo enzymatic hydrolysis to generate whey peptones, like Lactalbumin Hydrolysate. Whey peptones contain free amino acids and peptides, as well as carbohydrates, vitamins, and trace metals.

References

1. Dziuba, Babuchowski, Smoczynski and Smietana. 1999. Fractal analysis of caseinate structure. *Int Dairy J.* 9:287-292.
2. Huffman and Harper. 1999. Maximizing the value of milk through separation technologies. *J Dairy Sci.* 82:2238-2244.

Beef Extract Powder

Bacto Beef Extract, Desiccated

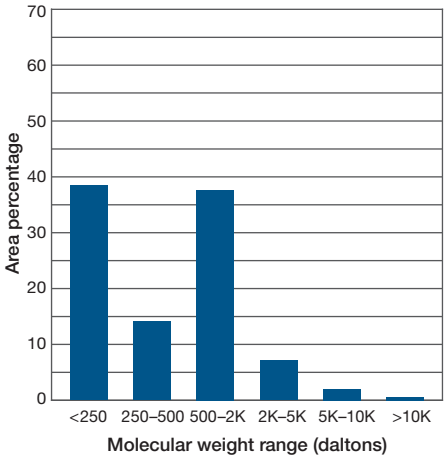
Difco Beef Extract

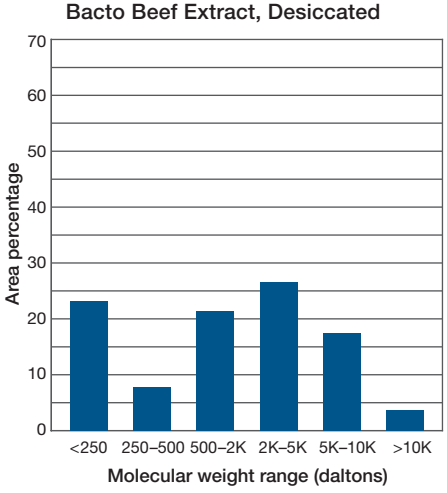
Product description

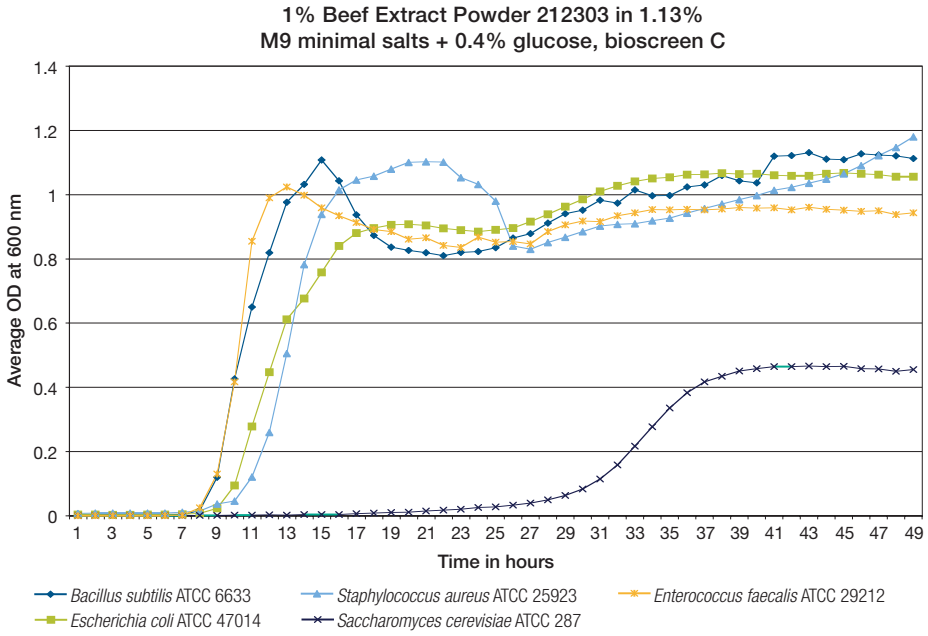
Beef Extract is derived from infusion of beef and provides a rich source of nutrients. The Beef Extract manufacturing process is different from those of many other peptones, so it can provide a unique blend of nutrients that can complement the properties of other peptones by contributing minerals, phosphates, energy sources, and other essential factors [1,2]. Beef Extract is a mixture of peptides and amino acids, nucleotide fractions, organic acids, minerals, and some vitamins. Beef Extract Powder is a meat extract dried to powder form. Bacto Beef Extract, Desiccated, is the dried form of Beef Extract paste.

Potential applications

Beef Extract is intended to replace aqueous infusion of meat in microbiological culture media. Beef Extract is frequently used at a concentration of 0.3 to 1.0% in culture media, although concentrations may vary depending on the nutritional requirements for the medium formulation.







Beef Extract was used in media for early studies of nonsporulating anaerobes of the intestinal tract and as a stock broth in the study of nutritional needs of streptococci. Prokofeva et al. [3] used Beef Extract for growing thermoacidophilic organisms newly isolated from hot springs in Kamchatka, Russia. Kataoka and Tokiwa [4] used Beef Extract as a nitrogen source in studies of mannose production by *Clostridium tertium* strains isolated from soil and methanogenic sludge. In addition, Beef Extract is a nutritive ingredient in many classical culture media, including antibiotic assay media described in the USP, and several media recommended for standard methods applications [5-7].

Physical characteristics

Beef Extract Powder is a light to medium cream to tan, free-flowing, homogeneous powder.

Bacto Beef Extract, Desiccated is a medium to dark brown, crystalline powder.

Difco Beef Extract is a medium to dark brown paste.

Ordering information

Product name	Size	Cat. No.
Beef Extract Powder	500 g	212303
Bacto Beef Extract, Desiccated	500 g	211520
Difco Beef Extract	500 g	212610

References

1. Cote. 1999. Media composition, microbial, laboratory scale. In Flickinger and Drew (ed.), Encyclopedia of Bioprocess Technology: Fermentation, Biocatalysis, and Bioseparation. John Wiley & Sons, Inc., New York.
2. Bridson and Brecker. 1970. Design and formulation of microbial culture media. In Norris and Ribbons (ed.), Methods in Microbiology, vol. 3A. Academic Press, New York.
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4. Kataoka and Tokiwa. 1998. Isolation and characterization of an active mannanase-producing anaerobic bacterium, *Clostridium tertium* KT-5A, from lotus soil. *J Appl Microbiol.* 84:357-367.
5. Rice, Baird, Eaton, Clesceri (ed.). 2012. Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association, Washington, D.C.
6. http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm.
7. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.

Gelysate Peptone

Product description

Gelysate Peptone is a pancreatic digest of gelatin, derived from porcine animal origin sources. Gelatin hydrolysate is high in glycine and proline residues [1]. Gelysate Peptone is characterized by low cystine, methionine, and tryptophan content.

Potential applications

Gelysate Peptone can be used for cultures requiring low carbohydrates, cystine, and tryptophan levels in cell culture and bacterial fermentation.

Physical characteristics

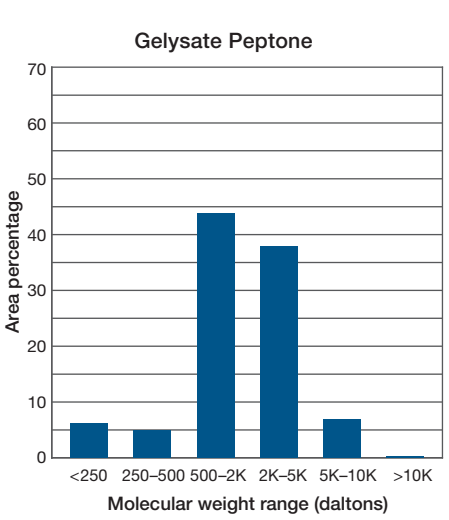
Gelysate Peptone is a fine, homogeneous powder, free of extraneous material.

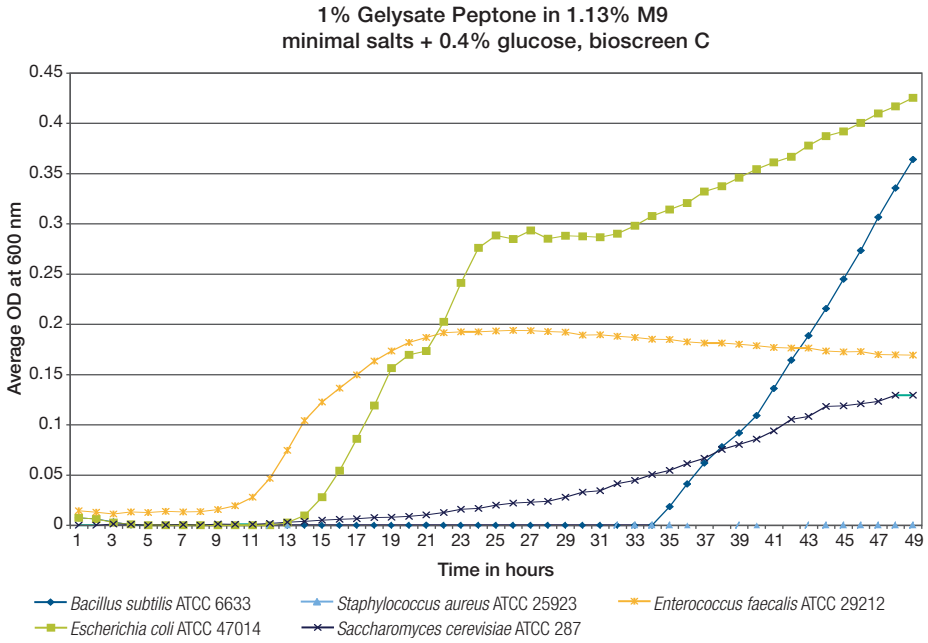
Ordering information

Product name	Size	Cat. No.
Gelysate Peptone	454 g	211870

Reference

1. Bridson and Brecker. 1970. Design and formulation of microbial culture media. In Norris and Ribbons (ed.), Methods in Microbiology, vol. 3A. Academic Press, New York.





Bacto Neopeptone

Product description

Bacto Neopeptone is an enzymatic digest of protein. Neopeptone contains a wide variety of peptide sizes in combination with vitamins and minerals.

Potential applications

Bacto Neopeptone is recommended for use in media for detection of fungi [1]. Apodaca and McKerrow [2] used Neopeptone for the cultivation of *Trichophyton rubrum* for the study of its proteolytic activity. Neopeptone has been cited as a component of culture media used for cultivation of human pathogens, notably *Bordetella pertussis* and group A streptococci.

Bacto Neopeptone has also been reported to provide nutrients for support of spirochetes and protozoa. Wyss et al. [3] used Neopeptone as a component of a medium for cultivation of *Treponema maltophilum* sp. nov., a fastidious oral anaerobe. Ifediba and Vanderberg [4] reported Neopeptone, in addition to calf serum, was used as an inexpensive replacement for human serum in the cultivation of *Plasmodium falciparum*, the causative agent of human malaria. Cushion and Ebbets [5] utilized Neopeptone in their investigations of various media for cultivating *Pneumocystis carinii* without feeder cells; optimal replication of *P. carinii* separated from host fungi cells was observed in media with Neopeptone and N-acetylglucosamine at low pH.

Physical characteristics

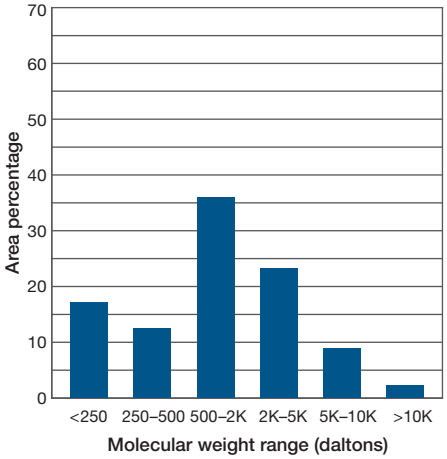
Bacto Neopeptone appears as tan, free-flowing, granules.

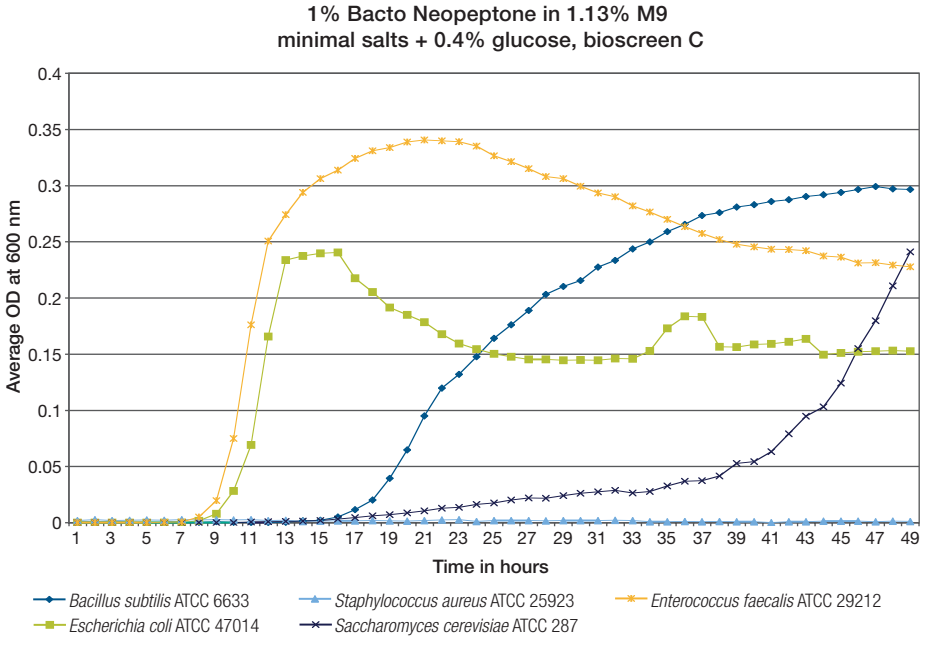
Ordering information

Product name	Size	Cat. No.
Bacto Neopeptone	500 g	211681
	10 kg	211680

References

1. Clesceri, Greenberg and Eaton (ed.). 1998. Standard methods for the examination of water and wastewater, 20th ed., 9-131-137. American Public Health Association, Washington, D.C.
2. Apodaca and McKerrow. 1990. Expression of proteolytic activity by cultures of *Trichophyton rubrum*. *J. Med. Vet. Mycol.* 28:159-171.
3. Wyss, Choi, Schupbach, Guggenheim and Gobel. 1996. Treponema maltophilum sp. nov., a small oral spirochete isolated from human periodontal lesions. *Int. J. Syst. Bacteriol.* 46:745-752.
4. Ifediba and Vanderberg. 1980. Peptones and calf serum as a replacement for human serum in the cultivation of *Plasmodium falciparum*. *J. Parasitol.* 66:236-239.
5. Cushion and Ebbets. 1990. Growth and metabolism of *Pneumocystis carinii* in axenic culture. *J. Clin. Microbiol.* 28:1385-1394.





Bacto Peptone

Product description

Bacto Peptone is an enzymatic digest of animal protein. This peptone was first introduced in 1914 and became the standard peptone for the preparation of bacteriological culture media. The nutritive value of Bacto Peptone is largely dependent on the amino acid content, which supplies essential nitrogen. Bacto Peptone contains only a negligible quantity of proteoses and more complex constituents.

Potential applications

Bacto Peptone is used as an organic nitrogen source in microbiological culture media for cultivation of a variety of bacteria and fungi. For example, Iwanaga et al. [1] utilized Bacto Peptone for production of cholera toxin by *Vibrio cholerae* O1 El Tor. Benkerroum et al. [2] reported using Bacto Peptone in a selective medium developed for isolating *Leuconostoc* species from food samples. Bacto Peptone was used in a culture medium for two anaerobic, extremely thermophilic archaea, *Thermococcus celer* and *Pyrococcus woesei*, by Blamey et al. [3].

Bacto Peptone has also been utilized as a nitrogen source in mammalian cell culture media formulations. Taylor et al. [4] used Bacto Peptone to supplement serum-free medium for several mammalian cell lines.

Sakoda and Fukusho [5] also utilized Bacto Peptone in serum-free culture for maintaining porcine kidney epithelial cells. Bacto Peptone is also useful as a supplement in mammalian cell culture with serum.

Researchers uncovered estrogenic activity associated with Bacto Peptone when observing that the estrone contained in Bacto Peptone was converted to estradiol by *Saccharomyces cerevisiae* after Bacto Peptone was added to the medium for culture of yeast. These findings suggest that adding estrogens to a medium containing Bacto Peptone for studies of estradiol production by yeast might confound results [6,7]. Bacto Peptone has also been used for the growth of the yeast *Pichia* for recombinant protein production [8], and for xylitol production [9].

Physical characteristics

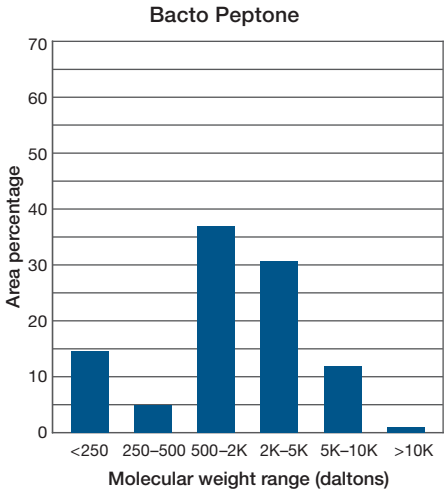
Bacto Peptone is a tan, free-flowing, homogeneous powder.

Ordering information

Product name	Size	Cat. No.
Bacto Peptone	500 g	211677
	2 kg	211820
	10 kg	211830

References

1. Iwanaga, Yamamoto, Higa, Ichinose, Nakasone and Tanabe. 1986. Culture conditions for stimulating cholera toxin production by *Vibrio cholerae* O1 El Tor. *Microbiol. Immunol.* 30:1075-1083.
2. Benkerroum, Misbah, Sandine and Elaraki. 1993. Development and use of a selective medium for isolation of Leuconostoc spp. from vegetables and dairy products. Appl. Environ. Microbiol. 59:607-609.
3. Blamey, Chiong, Lopez and Smith. 1999. Optimization of the growth conditions of the extremely thermophilic microorganisms *Thermococcus celer* and *Pyrococcus woesei*. *J. Microbiol. Methods* 38:169-175.
4. Taylor, Dworkin, Pumper and Evans. 1972. Biological efficacy of several commercially available peptones for mammalian cells in culture. *Exp. Cell Res.* 74:275-279.
5. Sakoda and Fukusho. 1998. Establishment and characterization of a porcine kidney cell line, FS-L3, which forms unique multicellular domes in serum-free culture. *In Vitro Cell. Dev. Biol. Anim.* 34:53-57.
6. Feldman and Krishnan. 1995. Estrogens in unexpected places: possible implications for researchers and consumers. *Environ. Health Perspect.* 103 Suppl 7:129-133.
7. Miller, Bottema, Stathis, Tokes and Feldman. 1986. Unexpected presence of estrogens in culture medium supplements: subsequent metabolism by the yeast *Saccharomyces cerevisiae.* Endocrinology 119:1362-1369.
8. Wang, Yang, Lin, Tsai, Wu and Mao. 2002. Expression, characterization, and purification of recombinant porcine lactoferrin in *Pichia* pastoris. *Protein Expression and Purification.* 25:41-49.
9. Jin, Cruz and Jeffries. 2005. Xylitol production by a *Pichia* stipites D-xylulokinase mutant. *Appl. Microbiol. Biotechnol.* 68(1):42-45.



Polypeptone Peptone

Product description

Polypeptone Peptone is a mixture of peptones made up of equal parts of pancreatic digest of casein and peptic digest of animal tissue. Polypeptone Peptone includes the high content of amino acids and small polypeptides characteristic of pancreatic digest of casein and the larger polypeptides characteristic of peptic digest of animal tissue.

Potential applications

Polypeptone Peptone has been found to meet nutritional requirements of various bacteria, fungi, and mammalian cells, for which a single source of casein or meat peptones has been unsatisfactory. Polypeptone Peptone has been utilized in culture media for the production of trypsin inhibitor by *Cephalosporium* sp.; [1] the production of bacterial cellulose by *Acetobacter* sp. A9; [2] the production of succinic acid from whey by *Anaerobiospirillum succiniciproducens*; [3] the mass production of luciferase-bacterial magnetic particles by recombinant *Magnetospirillum magneticum* AMB-1; [4] and the production of a novel tumor-killing factor by human macrophage-monocyte hybridomas [5].

Physical characteristics

Polypeptone Peptone is a fine homogeneous powder, free of extraneous material.

Ordering information

Product name	Size	Cat. No.
Polypeptone Peptone	454 g	211910
	10 kg	297108

References

1. Tsuchiya and Kimura. 1978. Production of trypsin inhibitor by a *Cephalosporium* sp. *Appl. Environ. Microbiol.* 35:631-635.
2. Son, Heo, Kim and Lee. 2001. Optimization of fermentation conditions for the production of bacterial cellulose by a newly isolated *Acetobacter* sp. A9 in shaking cultures. *Biotechnol. Appl. Biochem.* 33(Pt 1):1-5.
3. Lee, Lee, Kwon, Lee and Chang. 2000. Batch and continuous cultivation of *Anaerobiospirillum succiniciproducens* for the production of succinic acid from whey. *Appl. Microbiol. Biotechnol.* 54:23-27.
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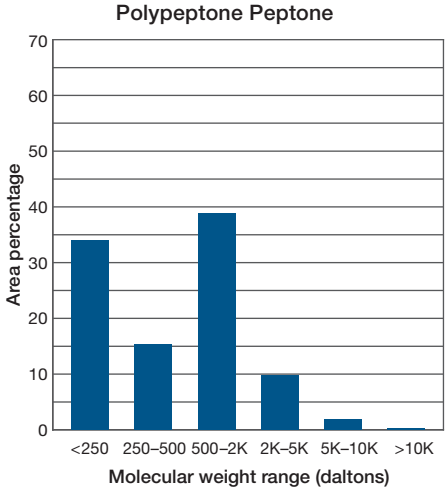
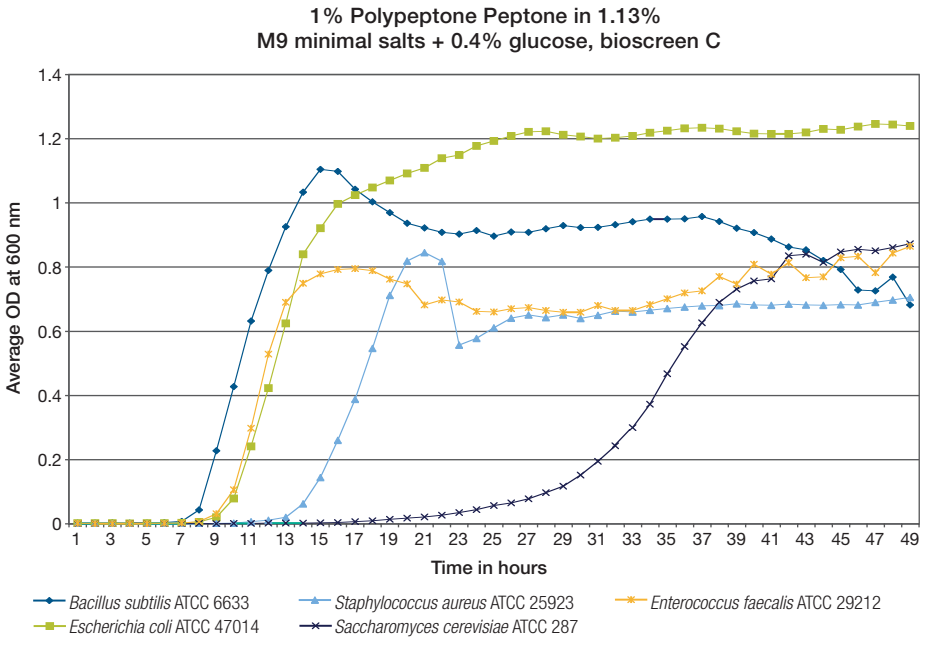
5. Taniyama, Yoshida and Furuta. 1988. Demonstration of a novel tumor-killing factor secreted from human macrophage-monocyte hybridomas. *J. Immunol.* 141:4061-4066.





Bacto Proteose Peptone
BiTek Proteose Peptone
Bacto Proteose Peptone No. 2
Bacto Proteose Peptone No. 3
BiTek Proteose Peptone No. 3
Bacto Proteose Peptone No. 4

Product description

Bacto Proteose Peptones are enzymatic digests of protein. Studies of peptic digests of animal tissue prepared under varying digestion parameters demonstrated that no single peptone is the most suitable nitrogen source for every microbiological application. These findings led to the development of Bacto Proteose Peptone, Bacto Proteose Peptone No. 2, and Bacto Proteose Peptone No. 3. Bacto Proteose Peptone No. 4 is a spray-dried version of Bacto Proteose Peptone.

BiTek Proteose Peptone and BiTek Proteose Peptone No. 3 are enzymatic digests of protein, developed to offer alternatives to Bacto Proteose Peptones for scale-up to production applications.

Potential applications

Bacto Proteose Peptone is used in preparing microbiological culture media and in producing bacterial toxins. Bacto Proteose Peptone was originally developed to produce a diphtheria toxin of high and uniform potency from cultures of *Corynebacterium diphtheriae*. Studies support the use of Bacto Proteose Peptone for production of diphtheria toxin, toxin-antitoxin mixtures, and toxoid.[1, 2] Bacto Proteose Peptone is also valuable in the production of other bacterial toxins: *Clostridium botulinum* toxin; [3] toxin from *Clostridium perfringens*; [4] toxin of hemolytic streptococci; [5] *Pneumococcus* toxin; [6] and toxin from *Salmonella pullorum* (*Salmonella choleraesuis* subsp. *choleraesuis*) [7].

Many factors account for the suitability of Bacto Proteose Peptone for the culture of fastidious pathogens, including the nitrogen components, buffering range, and the high content of proteoses. These elements create an environment beneficial to the maintenance of virulence and the elaboration of bacterial by-products. Thus, stock cultures are well preserved on media containing Bacto Proteose Peptone.

Bacto Proteose Peptone may be used in culture medium for a variety of applications, including the production of substances from the culture of bacteria, fungi, and mammalian cells. Bacto Proteose Peptone has been utilized in a medium for producing glycosidases from *Bacteroides fragilis* [8] and to stimulate amyloglucosidase production by *Aspergillus* species [9]. It has been used to cultivate halophilic bacteria isolated from soil in Egypt for production of polymers [10]. Jan et al. [11] reported that Bacto Proteose Peptone as supplementation to defined medium resulted in significant increases in cell number and specific monoclonal antibody production in a batch mammalian culture system. Bacto Proteose Peptone has also been used to provide nutrients for axenic culture of amoeba [12].

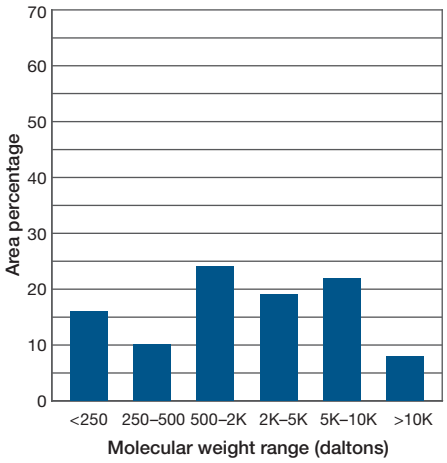
BiTek Proteose Peptone was developed to provide an alternative product to Bacto Proteose Peptone, with similar growth characteristics.

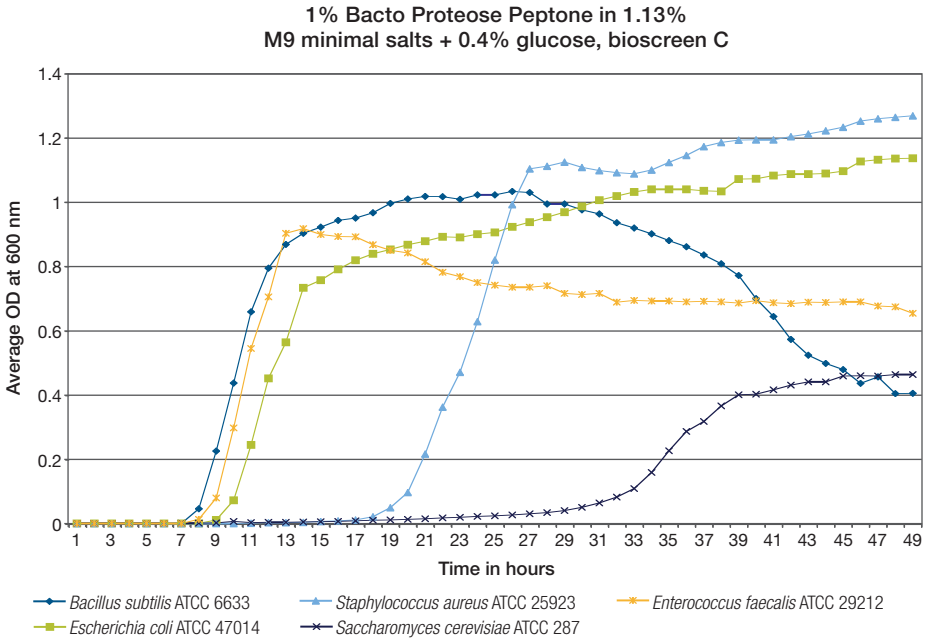
Bacto Proteose Peptone No. 2 is used in preparing microbiological culture media. It was originally developed for use in media for the production of diphtheria toxin. Bunney and Thomas [13] reported good yield of diphtheria toxin with Bacto Proteose Peptone No. 2 in a simple peptone-sugar-sodium acetate medium.

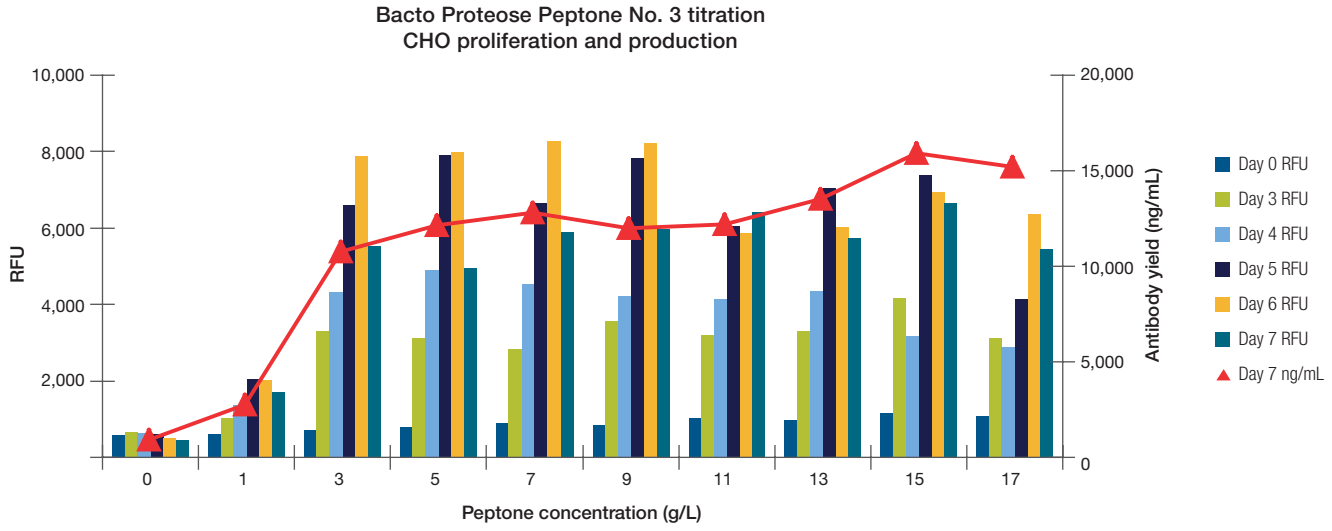
Bacto Proteose Peptone No. 3 is used in preparing microbiological and mammalian cell culture media. It provides superior nutrition for fastidious microorganisms, supporting growth of streptococci, staphylococci, pneumococci, gonococci, *Neisseria* spp., *C. diphtheriae*, and other organisms that require a highly nutritious substrate. For example, Ifediba and Vanderberg [14] reported that Bacto Proteose Peptone No. 3, in addition to calf serum, was used as an inexpensive replacement for human serum in cultivation of *Plasmodium falciparum*, the causative agent of human malaria. Mammalian cell culture manufacturers have found significant yield improvements in using Bacto Proteose Peptone No. 3. Bacto Proteose Peptone No. 3 has also been shown to give significant yield (antibody production) improvements, when used for cell culture of CHO cells [15].

BiTek Proteose Peptone No. 3 was developed to provide an alternative product to Bacto Proteose Peptone No. 3, with similar growth characteristics.

Bacto Proteose Peptone No. 4 is a spray-dried version of Bacto Proteose Peptone. It offers the same beneficial nutrients as Bacto Proteose Peptone for growth promotion and toxin production in a wide range of fastidious microorganisms.







Physical characteristics

Bacto Proteose Peptone appears as tan, free-flowing granules.

BiTek Proteose Peptone is a tan, free-flowing, homogeneous powder.

Bacto Proteose Peptone No. 2 appears as tan, free-flowing granules.

Bacto Proteose Peptone No. 3 appears as golden tan, free-flowing granules.

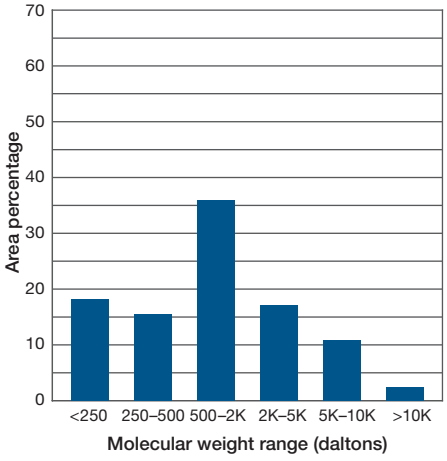
BiTek Proteose Peptone No. 3 is a light beige, free-flowing, homogeneous powder.

Bacto Proteose Peptone No. 4 is a light beige, free-flowing, homogeneous powder.

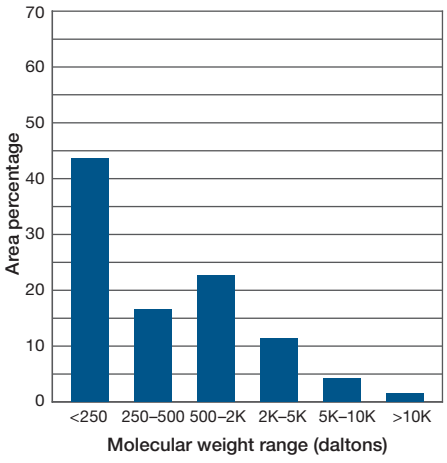
Ordering information

Product name	Size	Cat. No.
Bacto Proteose Peptone	500 g	211684
	10 kg	212010
BiTek Proteose Peptone	10 kg	253310
Bacto Proteose Peptone No. 2	500 g	212120
	10 kg	212110
Bacto Proteose Peptone No. 3	500 g	211693
	2 kg	212220
	10 kg	212230
	50 kg	211692
BiTek Proteose Peptone No. 3	25 kg	253720
Bacto Proteose Peptone No. 4	10 kg	211715

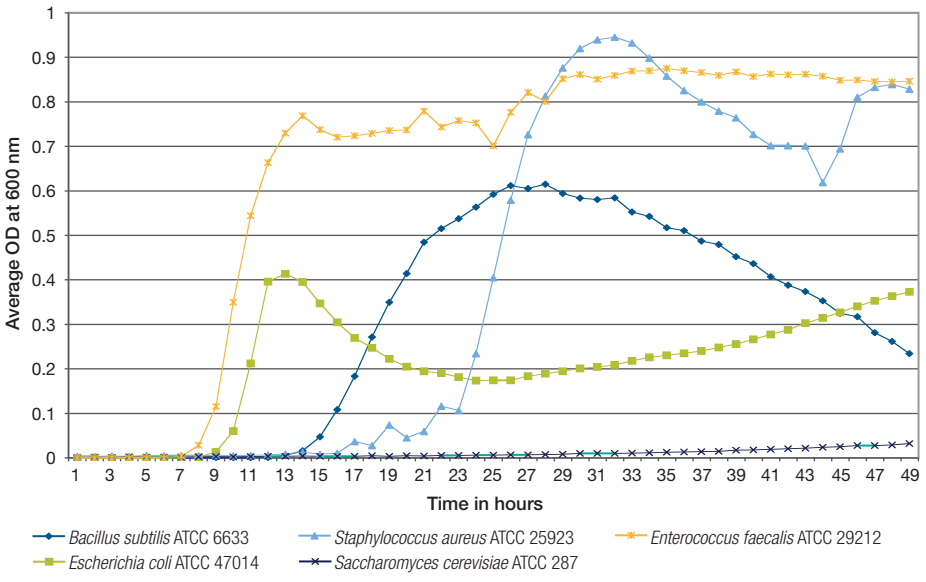
BiTek Proteose Peptone



Bacto Proteose Peptone No. 2



1% Bacto Proteose Peptone No. 2 in 1.13% M9 minimal salts + 0.4% glucose, bioscreen C



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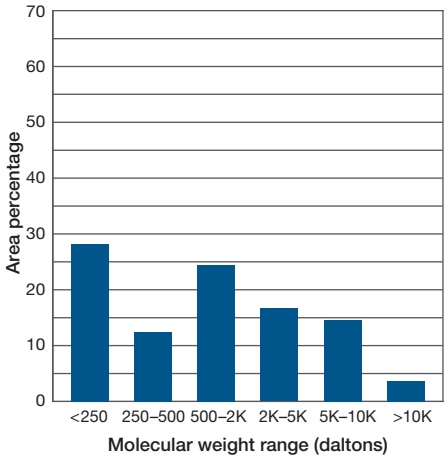
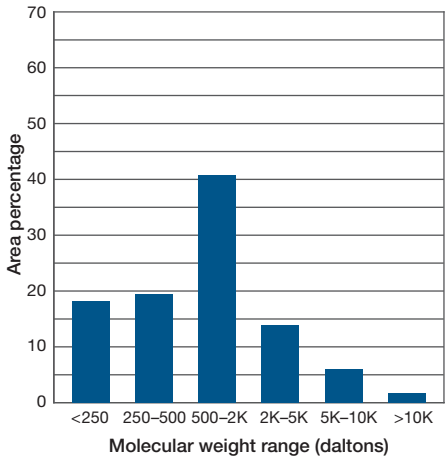
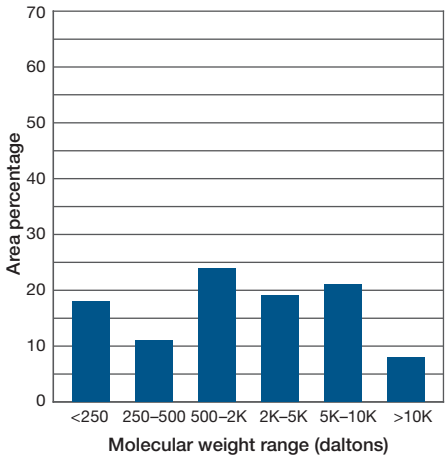


BiTek Proteose Peptone No. 3



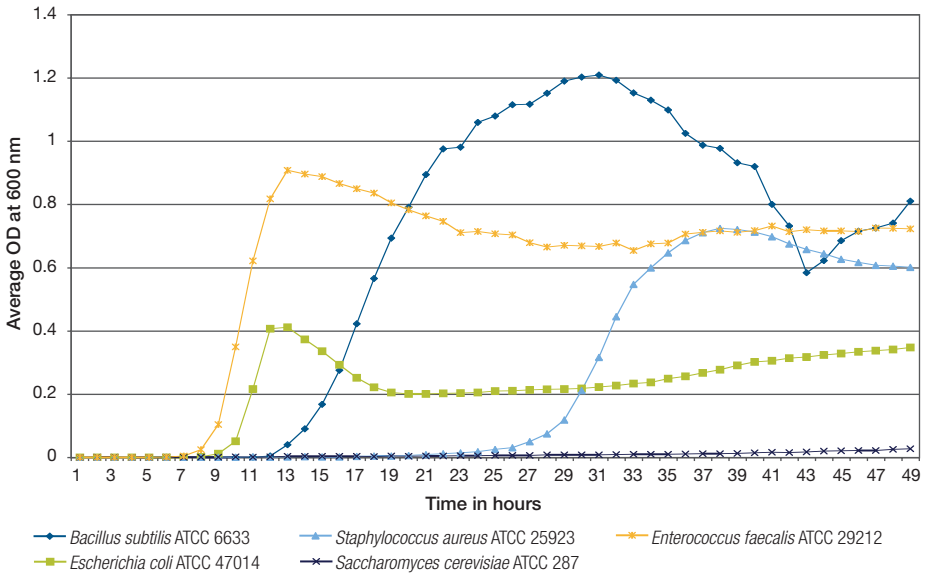
Bacto Proteose Peptone No. 4



References

1. Kirkbride, Berthelsen and Clark. 1931. Comparative studies of infusion and infusion-free diphtheria toxin in antitoxin production and in standardization by the flocculation, subcutaneous, and intracutaneous tests. *J. Immunol.* 21:1-20.
2. Hazen and Heller. 1931. Further studies upon the effect of various carbohydrates on production of diphtheria toxin with special reference to its flocculating titer and final pH. *J. Bacteriol.* 23:195-209.
3. Nelson. 1927. The relationship between the intracellular globulin and the toxin of *C. botulinum*. *J. Infect. Dis.* 41:9-12.
4. Mollby and Holme. 1976. Production of phospholipase C (alpha-toxin), haemolysins and lethal toxins by Clostridium perfringens types A to D. *J. Gen. Microbiol.* 96:137-144.
5. Kirkbride and Wheeler. 1926. Studies of the toxins of the hemolytic streptococci associated with scarlet fever. *J. Immunol.* 11:477-497.
6. Kneeland and Dawes. 1932. Studies on the common cold: V. The relationship of pathogenic bacteria to upper respiratory diseases in infants. *J. Exp. Med.* 55:735-744.
7. Hanks and Rettger. 1931. Bacterial endotoxin; search for a specific intracellular toxin in *S. pullorum*. *J. Immunol.* 22:283-314.
8. Berg, Nord and Wadstrom. 1978. Formation of glycosidases in batch and continuous culture of *Bacteroides fragilis*. *Appl. Environ. Microbiol.* 35:269-273.
9. Mamo and Gessesse. 1999. Production of raw-starch digesting amyloglucosidase by Aspergillus sp. GP-21 in solid state fermentation. J. *Ind. Microbiol. Biotechnol.* 22:622-626.
10. Hezayen, Rehm, Eberhardt and Steinbuchel. 2000. Polymer production by two newly isolated extremely halophilic archaea: application of a novel corrosion-resistant bioreactor. *Appl. Microbiol. Biotechnol.* 54:319-325.
11. Jan, Jones, Emery and Al-Rubeai. 1994. Peptone, a low-cost growth-promoting nutrient for intensive animal cell culture. *Cytotechnol.* 16:17-26.
12. Shukla, Kaul and Mehlotra. 1989. Development of improved media for axenic cultivation of *Acanthamoeba culbertsoni*, Singh and Das 1970. Indian J. Exp. Biol. 27:785-791.
13. Bunney and Thomas. 1936. Diphtheria toxin-production on broths made from dried complete media. *J. Immunol.* 31:95-102.
14. Ifediba and Vanderberg. 1980. Peptones and calf serum as a replacement for human serum in the cultivation of *Plasmodium falciparum*. *J. Parasitol.* 66:236-239.
15. Chaturvedi, Sun, O'Brien, Liu and Brooks. 2014. Comparison of the behavior of CHO cells during cultivation in 24-square deep well microplates and conventional shake flask systems. *Biotechnology Reports.* 1-2:22-26.

1% Bacto Proteose Peptone No. 3 in 1.13% M9 minimal salts + 0.4% glucose, bioscreen C



Bacto Tryptose

Product description

Bacto Tryptose is a mixed enzymatic hydrolysate with distinctive nutritional properties. The digestive process of Tryptose results in assorted peptides of a higher molecular weight suitable for long-chain amino acid requirements.

Potential applications

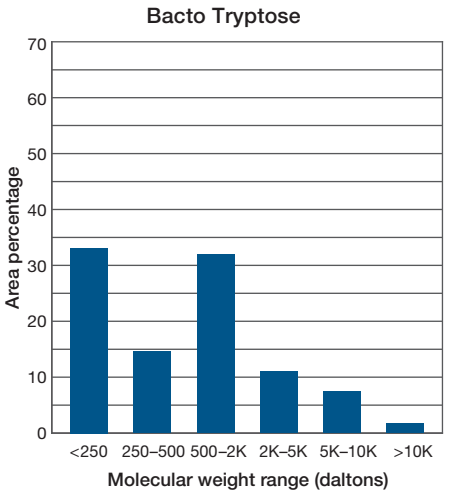
Bacto Tryptose was originally developed as a peptone particularly adapted to the growth requirements of *Brucella*. Tryptose is very useful for cultivation of streptococci, pneumococci, meningococci, and other fastidious organisms, and was found to be superior to meat infusion peptone media previously used for these organisms [1,2]. Mobley et al. [3] reported that tryptose broth was the preferred medium for strains of *Bordetella bronchiseptica* in studies of phosphatase activity.

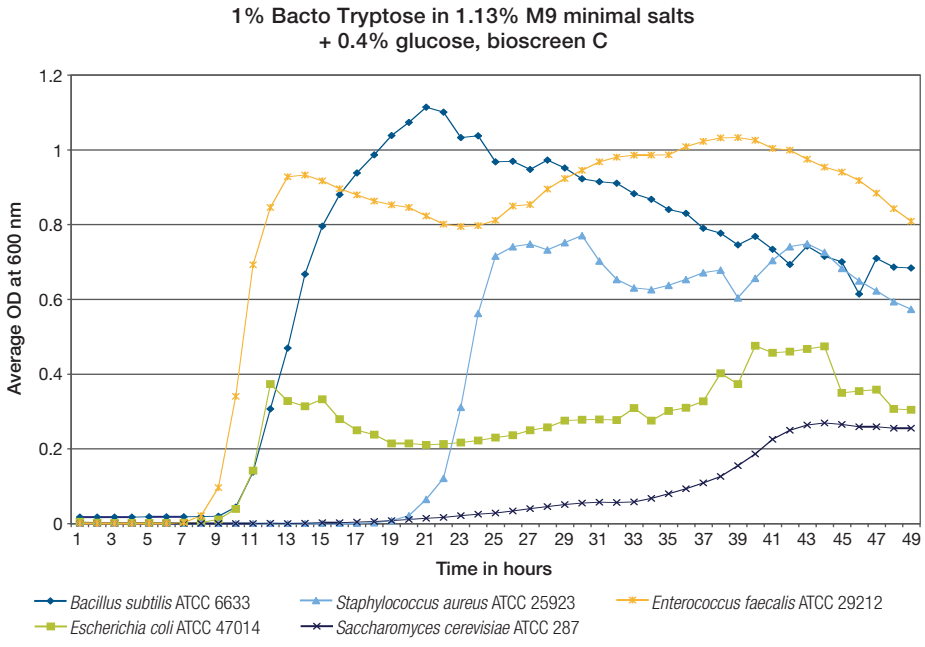
Bacto Tryptose has been reported as beneficial for cell culture applications. Litwin [4] found Tryptose suited to supplementing a serum-free medium for growing human diploid fibroblasts. Vaughn and Fan [5] established that Tryptose provided free amino acids necessary for growth of *Spodoptera frugiperda* and *Lymantria dispar* insect cell lines. Bacto Tryptose is often used as a biomass enhancer for recombinant *Escherichia coli* production.

Tryptose is the major ingredient of tryptose phosphate broth (TPB), an often-used medium for various culture applications. Hata and Kojima [6] have shown TPB to be a useful supplement for *in vitro* culture of the nematode *Angiostrongylus cantonensis*. TPB was also reported as a supplement to a medium for cultivating a protozoan parasite, which parasitizes vectors of Chagas disease, on its insect cell host [7]. *Spodoptera frugiperda*, a cotton pest in Argentina [8], and several tick cell lines have also been grown using a TPB-supplemented medium [9]. TPB has been reported as a suitable supplement for the growth of baby hamster kidney (BHK) cells [10] and porcine kidney cells [11].

Physical characteristics

Bacto Tryptose appears as tan, free-flowing granules.





Ordering information

Product name	Size	Cat. No.
Bacto Tryptose	500 g	211713
	10 kg	211709

References

1. Casman. 1942. A dehydrated medium to supplement meat infusion as a base for blood agar. *J Bacteriol.* 43:33.
2. Casman. 1947. A noninfusion blood agar base for neisseriae, pneumococci and streptococci. *Am J Clin Pathol.* 17:281-289.
3. Mobley, Chengappa, Kadel and Stuart. 1984. Effect of pH, temperature and media on acid and alkaline phosphatase activity in “clinical” and “nonclinical” isolates of *Bordetella* bronchiseptica. *Can J Comp Med.* 48:175-178.
4. Litwin. 1985. Further studies on a tryptose based serum-free medium for human diploid fibroblasts. *Dev Biol Stand.* 60:25-33.
5. Vaughn and Fan. 1997. Differential requirements of two insect cell lines for growth in serum-free medium. *In Vitro Cell Dev Biol Anim.* 33:479-482.
6. Hata and Kojima. 1990. *Angiostrongylus cantonensis*: *in vitro* cultivation from the first-stage to infective third-stage larvae. *Exp Parasitol.* 70:467-482.
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8. Deutschmann and Jager. 1994. Optimization of the growth conditions of Sf21 insect cells for high-density perfusion culture in stirred-tank bioreactors. *Enzyme Microb Technol.* 16:506-512.
9. Munderloh and Kurtti. 1989. Formulation of medium for tick cell culture. *Exp Appl Acarol.* 7:219-229.
10. Prodafikas and Plavsic. 2000. Effects of medium supplements on BHK-21 cell growth and bluetongue virus production. *Focus* 22:35.
11. Sakoda and Fukusho. 1998. Establishment and characterization of a porcine kidney cell line, FS-L3, which forms unique multicellular domes in serum-free culture. *In Vitro Cell Dev Biol Anim.* 34:53-57.

Acidicase Peptone

Bacto Casamino Acids

Bacto Casamino Acids, Technical

Difco Casamino Acids, Vitamin Assay

Product description

Acidicase Peptone is a hydrochloric acid hydrolysate of casein. The manufacturing process produces a casein peptone which has a high salt content of approximately 37% and nitrogen content of approximately 8%. The hydrolysis of the casein, a milk protein rich in amino acid nitrogen, is carried out until all the nitrogen is converted to amino acids or other compounds of relative simplicity. Casein contains little cystine and is deficient in tryptophan, which is destroyed by the acid treatment.

Bacto Casamino Acids is an acid hydrolysate of casein, prepared according to the method described by Mueller and Miller [1]. The method described reduces the sodium chloride and iron content of the hydrolyzed casein. This hydrolyzed casein, supplemented with inorganic salts, growth factors, cystine, maltose, and an optimum amount of iron, was used by Mueller and Miller to prepare diphtheria toxin. Bacto Casamino Acids duplicate this specially treated hydrolyzed casein.

Bacto Casamino Acids, Technical is prepared similarly to Bacto Casamino Acids but is a less refined product, leaving a higher sodium chloride and iron content than in Bacto Casamino Acids.

Difco Casamino Acids, Vitamin Assay is an acid digest of casein specially treated to markedly reduce or eliminate certain vitamins. It is recommended for use in microbiological assay media and in studies of the growth requirements of microorganisms.

Potential applications

Acidicase Peptone is intended for use as a nutritional supplement in vitamin assays, susceptibility testing, and other laboratory media and microbial fermentation in which the high salt content will not interfere.

Bacto Casamino Acids, due to the nearly complete hydrolysis of casein and the low sodium chloride and iron content, makes an excellent supplement for many media formulations. It has been recommended as a compromise for the replacement of pure amino acids in a defined medium for the growth of *Lactobacillus*, thus eliminating the complexity of preparation [2]. Additionally, it has been successfully used, along with Bacto Tryptone, in nutritional studies to determine a bacterium's growth requirement for peptides or amino acids [3,4]. It also works well as a component in laboratory media. It has been utilized in such diverse applications as TYI-S-33 media for the parasite *Entamoeba histolytica* and LCM medium for the growth of a nematode–bacterium complex [5].

Bacto Casamino Acids, Technical provides similar benefits to Bacto Casamino Acids, for applications requiring a less refined hydrolysate.

Difco Casamino Acids, Vitamin Assay is commonly used as the amino acid source in early phases of nutrition work [6]. Casamino Acids, Vitamin Assay was used as the acid hydrolyzed casein in studies on *p*-aminobenzoic acid and *p*-teroylglutamic acid as growth factors for *Lactobacillus* species [7].

Physical characteristics

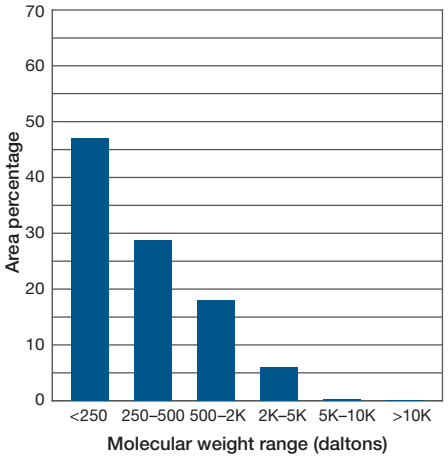
Acidicase Peptone is a fine, homogeneous powder, free of extraneous material.

Bacto Casamino Acids is a very light beige to tan, homogeneous, free-flowing powder.

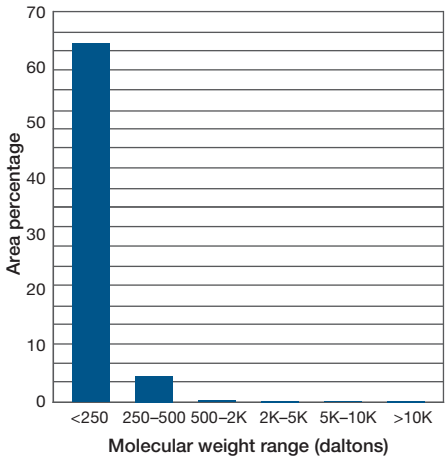
Bacto Casamino Acids, Technical, is a very light beige, homogeneous, free-flowing powder.

Difco Casamino Acids, Vitamin Assay, is a light beige, homogeneous, free-flowing powder.

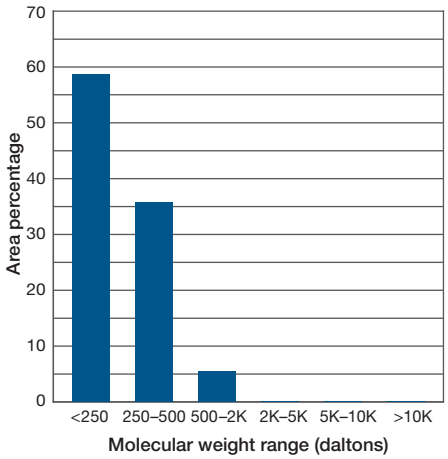
Acidicase Peptone



Bacto Casamino Acids



Bacto Casamino Acids, Technical



Ordering information

Product name	Size	Cat. No.
Acidicase Peptone	500 g	211843
Bacto Casamino Acids	500 g	223050
	2 kg	223020
	10 kg	223030
Bacto Casamino Acids, Technical	500 g	223120
	10 kg	223110
Difco Casamino Acids, Vitamin Assay	100 g	228820
	500 g	228830

References

1. Mueller and Miller. 1941. Production of diphtheria toxin of high potency (100 If) on a reproducible medium. *J Immunol.* 40:21-32.
2. Van Niel and Hahn-Hägerdal. 1999. Nutrient requirements of lactococci in defined growth media. *Appl Microbiol Biotechnol.* 52:617-627.
3. Takahashi, Sato and Yamada. 2000. Metabolic pathways for cytotoxic end product formation from glutamate- and aspartate containing peptides by Porphyromonas gingivalis. *J Bacteriol.* 182:4704-4710.
4. Attwood, Klieve, Ouwerkerk and Patel. 1998. Ammonia-hyperproducing bacteria from New Zealand ruminants. *Appl Environ Microbiol.* 64:1796-1804.
5. Strauch and Ehlers. 2000. Influence of the aeration rate on the yields of the biocontrol nematode Heterorhabditis megidis in monoxenic liquid cultures. *Appl Microbiol Biotechnol.* 54:9-13.
6. Nolan. 1971. *Mycol.* 63:1231.
7. Sarett. 1947. *J Biol Chem.* 171:265.

Biosate Peptone

Product description

Biosate Peptone is a mixed hydrolysate comprised of 65% pancreatic digest of casein and 35% yeast extract.

Potential applications

Biosate Peptone can be used as a component in microbiological media or in fermentation applications. The synergistic effect of two or more types of hydrolysates is well documented and has been utilized for decades in culture media formulation. The combination of pancreatic digest of casein and yeast extract provides nutritional benefits that are not provided by the components alone. It has been reported that the combined use of these two peptones has improved toxin production in *Clostridia* [1,2]. Additionally, the combination of pancreatic digest of casein and yeast extract has been used successfully as components in media which supported the first-time culturing of a nematode without the need for its symbiotic bacteria [3].

Physical characteristics

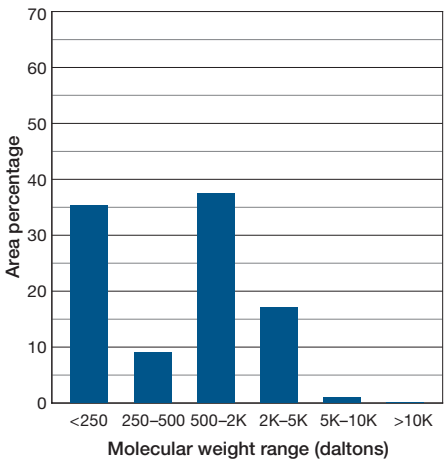
Biosate Peptone is a fine, homogeneous, free-flowing powder, free of extraneous material.

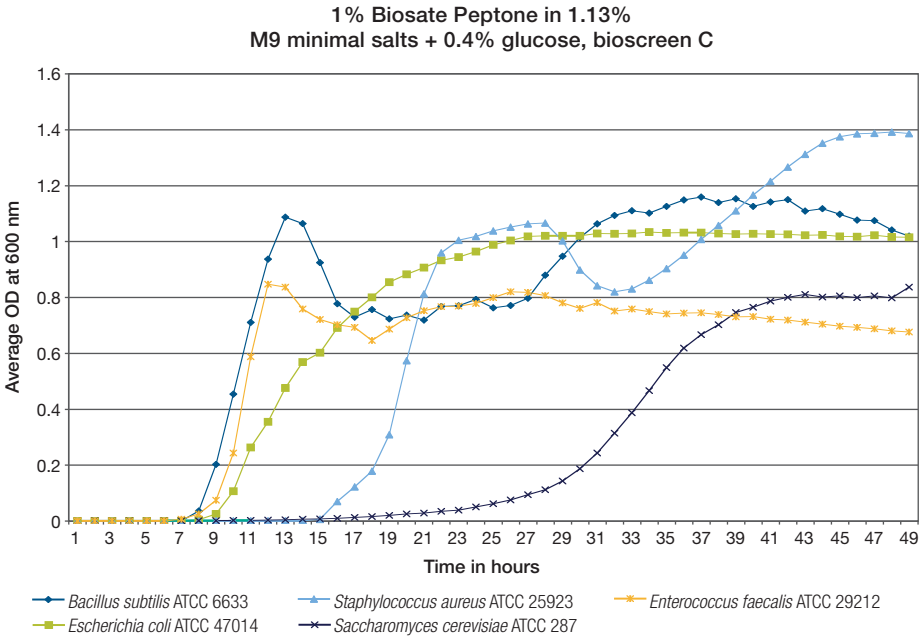
Ordering information

Product name	Size	Cat. No.
Biosate Peptone	454 g	211862
	25 lb (11.3 kg)	294312

References

1. Artemenko, Ivanova, Nenashev, Kuznetsova and Ochkina. 1985. Use of experimental analytical method for equilibrating nutrient broths for *Clostridium perfringens* type A growth and toxin production. *Zh Mikrobiol Epidemiol Immunobiol.* 11:37-41.
2. Siegel and Metzger. 1980. Effect of fermentation conditions on toxin production by Clostridium botulinum type B. *Appl Environ Microbiol.* 40:1023-1026.
3. Dorsman and Bijl. 1985. Cultivation of free-living stages of *Trichostrongylus colubriformis* in media without bacteria, animal tissue extract, or serum. *J Parasitol.* 71:200-203.





Difco Casein Digest

Product description

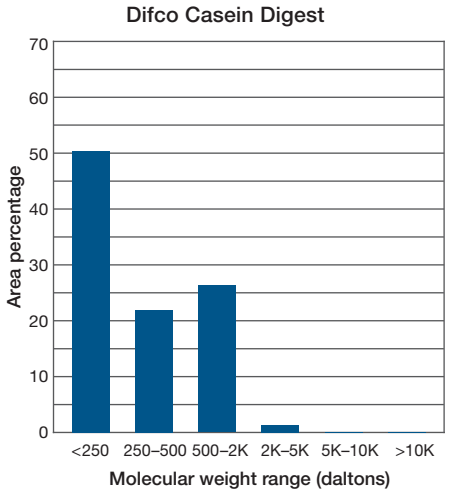
Difco Casein Digest is an enzymatic digest of casein. Difco Casein Digest is hydrolyzed under conditions different from other enzymatic digests of casein such as Bacto Tryptone and Bacto Casitone.

Potential applications

Difco Casein Digest can be used as a component in microbiological culture media. It was developed for use in molecular genetics media and can be used to make NZCYM broth, NZYM broth, and NZM broth, which are used for cultivating recombinant strains of *Escherichia coli* [1].

Physical characteristics

Difco Casein Digest is a light beige, homogeneous, free-flowing powder.



Ordering information

Product name	Size	Cat. No.
Difco Casein Digest	500 g	211610

Reference

1. Ausubel, Brent, Kingston, Moore, Seidman, Smith and Struhl (ed.). 1994. Current Protocols in Molecular Biology, vol. 1. Current Protocols, New York, N.Y.

Bacto Casitone

Difco Tryptone

Bacto Tryptone

BiTek Tryptone

Product description

Bacto Casitone is a pancreatic digest of casein. The manufacturing process for an enzymatic digest of casein is not as destructive as an acid hydrolysis. Thus, the casein is not broken down as completely into its constituent components. In many cases, this makes for a more nutritious hydrolysate, especially for those organisms that prefer peptides to amino acids.

Difco Tryptone is a pancreatic digest of casein and is the primary nitrogen source in trypticase soy broth and agar.

Bacto Tryptone is a pancreatic digest of casein. It was developed while investigating a peptone particularly suitable for the elaboration of indole by bacteria.

BiTek Tryptone is prepared similarly to Bacto Tryptone, but the final product goes through fewer refinement steps during processing.

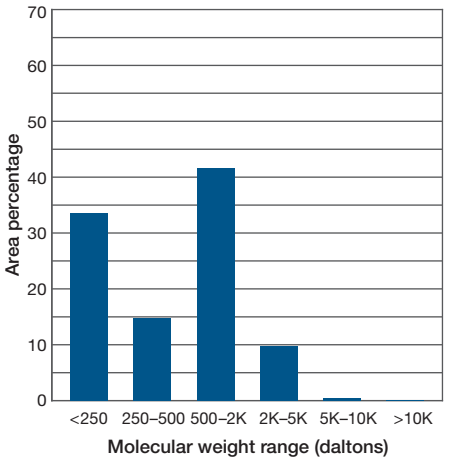
Potential applications

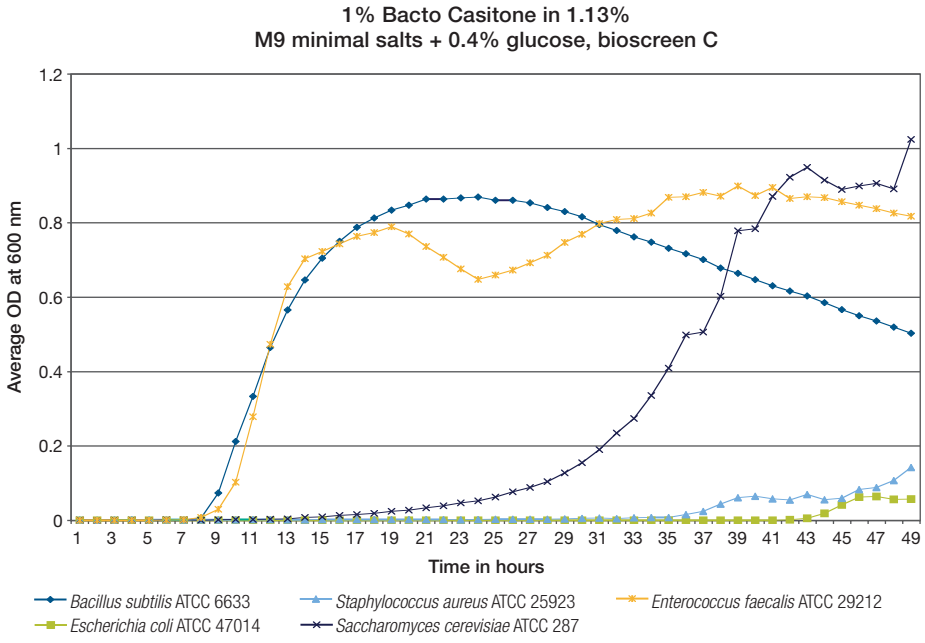
Bacto Casitone can be used as a component in microbiological media or in fermentation applications. The stability of lyophilized influenza virus vaccine has been shown to be augmented by the addition of 2% casitone [1].

Difco Tryptone is recommended for use in media formulations when good growth of fungi and bacteria is required. It is referenced in Official Methods of Analysis of AOAC International [2].

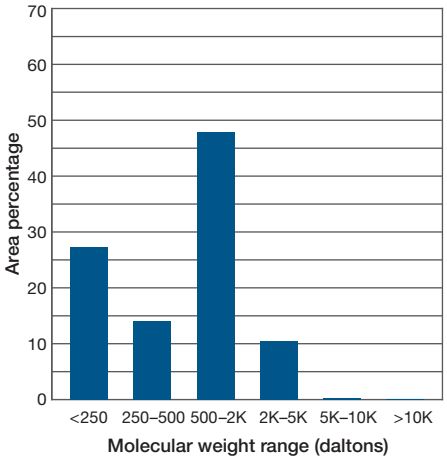
Bacto Tryptone has been used in conjunction with casamino acids in nutritional studies to determine amino acids vs peptide utilization [3,4]. Bacto Tryptone also works well in fermentation applications. It has been used successfully with common organisms such as *Escherichia coli* [5], as well as uncommon organisms such as the diatom *Nitzschia laevis* [6].

BiTek Tryptone provides some of the same benefits as Bacto Tryptone in instances when a less refined hydrolysate can be utilized.

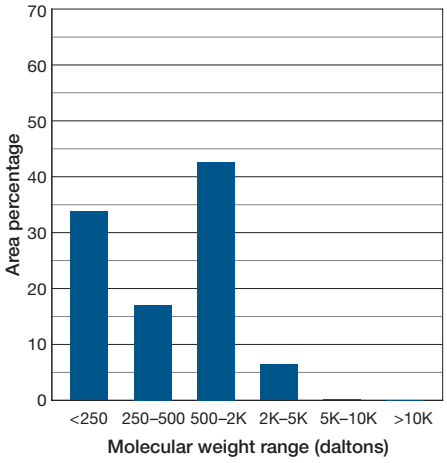




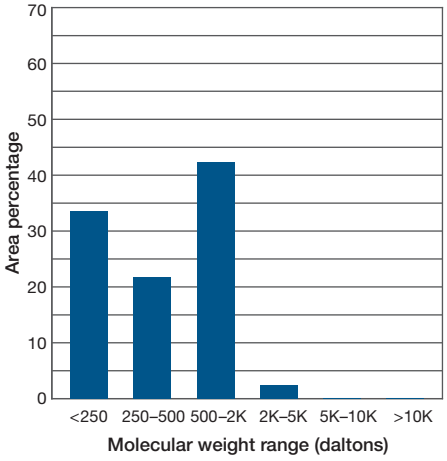
Difco Tryptone



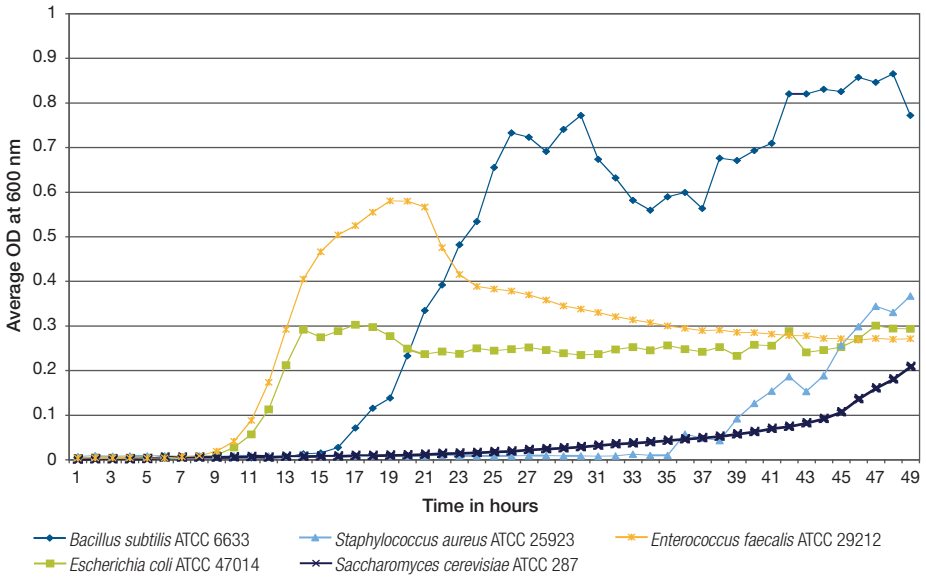
Bacto Tryptone



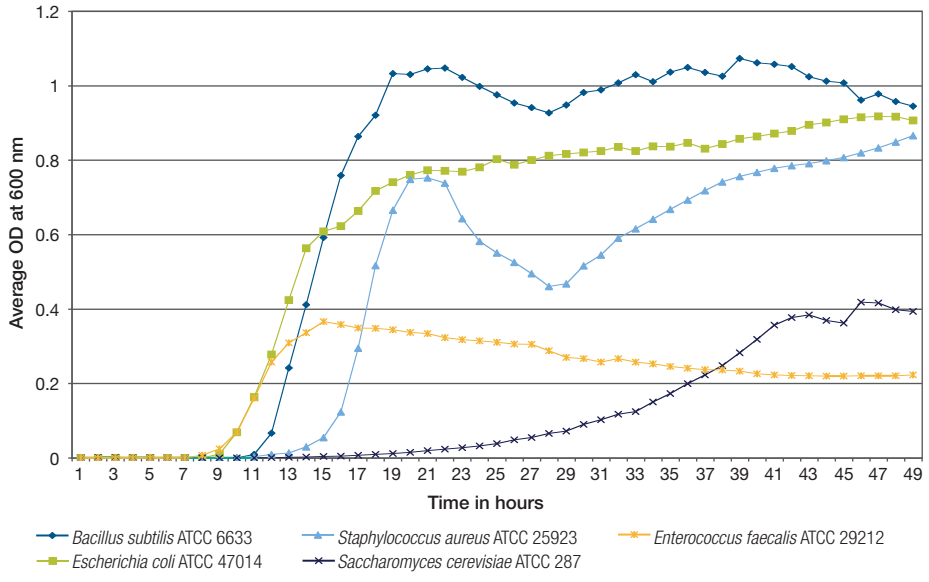
BiTek Tryptone



1% Difco Tryptone in 1.13%
M9 minimal salts + 0.4% glucose, bioscreen C



1% Bacto Tryptone in 1.13%
M9 minimal salts + 0.4% glucose, bioscreen C



Physical characteristics

Bacto Casitone appears as tan, free-flowing granules.

Difco Tryptone is a fine, homogeneous powder, free of extraneous material.

Bacto Tryptone is a light beige, homogeneous, free-flowing powder.

BiTek Tryptone is a light beige, homogeneous, free-flowing powder.

Ordering information

Product name	Size	Cat. No.
Bacto Casitone	500 g	225930
	10 kg	225910
Difco Tryptone	454 g	211921
	5 lb (2.3 kg)	211922
	25 lb (11.3 kg)	211923
Bacto Tryptone	500 g	211705
	2 kg	211699
	10 kg	211701
BiTek Tryptone	10 kg	251420

References

1. Yannarell, Goldberg and Hjorth. 2002 Stabilizing cold-adapted influenza virus vaccine under various storage conditions. *J Virol Methods.* 102(1-2):15-25.
2. Horowitz (ed.). 2005. Official Methods of Analysis of AOAC International. 18th ed. AOAC International, Gaithersburg, MD.
3. Takahashi and Yamada. 2000. Metabolic pathways for cytotoxic end product formation from glutamate- and aspartate-containing peptides by *Porphyromonas gingivalis. J Bacteriol.* 182:4704-4710.
4. Nagel, Oostra, Tramper and Rinzema. 1999. Improved model system for solid-substrate fermentation: effects of pH, nutrients and buffer on fungal growth rate. *Process Biochem.* 35:69-75.
5. Sivakesavs, Chen, Hackett, Huang, Lam, Lam, Siu, Wong and Wong. 1999. Production of excreted human epidermal growth factor (hEGF) by an efficient recombinant *Escherichia coli* system. *Process Biochem.* 34:893-900.
6. Wen and Chen. 2001. Optimization of nitrogen sources for heterotrophic production of eicosapentanoic acid by the diatom Nitzschia laevis. *Enzyme Microbial Technol.* 29:341-347.

Bacto TC Lactalbumin Hydrolysate

Product description

Bacto TC Lactalbumin Hydrolysate is the enzymatically hydrolyzed protein portion of milk whey, which is recognized as a complete protein source. This product is a mixture of peptides, amino acids, and carbohydrates, both simple and complex.

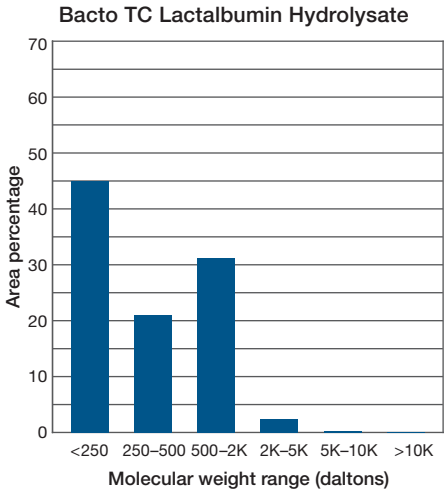
Potential applications

Bacto TC Lactalbumin Hydrolysate is intended as a nutritional supplement for bacterial, insect, and mammalian cell culture. For years, Bacto TC Lactalbumin Hydrolysate has been used as a nutritional source for *Lactobacillus*. It is also useful for indole testing because of its tryptophan content.

Bacto TC Lactalbumin Hydrolysate is frequently used in mammalian cell culture media as an amino acid supplement [1].

Physical characteristics

Bacto TC Lactalbumin Hydrolysate is a buff to tan, homogeneous, free-flowing powder.



Ordering information

Product name	Size	Cat. No.
Bacto TC Lactalbumin Hydrolysate	500 g	259962
	10 kg	259961

Reference

1. Bridson and Brecker. 1970. Design and formulation of microbial culture media. In Norris and Ribbons (ed.), Methods in Microbiology, vol. 3A. Academic Press, New York.

Typical analyses: Animal origin peptones

Meat peptones

Gibco product	Total nitrogen (%)	Amino nitrogen (%)	AN/TN	Total carbohydrate (mg/g)	Ash (%)	Loss on drying (%)	NaCl (%)	pH (1% solution)	Calcium (µg/g)	Iron (µg/g)	Magnesium (µg/g)	Potassium (µg/g)	Sodium (µg/g)	Chloride (%)	Sulfate (%)	Phosphate (%)	Alanine (% free)	Arginine (% free)	Asparagine (% free)	Aspartic acid (% free)	Cystine (% free)	Glutamic acid (% free)	Glutamine (% free)	
Beef Extract Powder	12.4	2.3	0.19	56.1	9.3	3.5	0.3	6.9	264	27.4	285	28793	18510	0.00	0.53	3.22	1.8	2.8	0.6	0.6	0.2	2.5	0.1	
Bacto Beef Extract, Desiccated	13.9	2.0	0.14	9.8	7.7	1.8	1.7	6.9	53	19.2	92	31423	21645	1.62	0.70	0.43	1.1	1.3	0.1	0.3	0.0	0.6	0.0	
Gelysate Peptone	17	2.9	0.17	11.58	3.8	4.9	0.2	6.9	381	11.8	150	656	11090	0.00	1.66	0.18	0.8	3.1	0.1	0.1	0.3	0.2	0.1	
Bacto Neopeptone	13.6	3.2	0.2	13.13	6.9	4.0	1.4	7.4	77	5.3	28	8945	36313	0.48	0.45	2.59	0.5	0.5	0.2	0.3	0.4	0.6	0.0	
Bacto Peptone	15.4	3.5	0.2	6.29	3.8	2.7	1.7	7.1	30	7.8	17	2487	18127	0.9	0.32	0.40	1.2	2.8	0.3	0.3	0.0	0.7	0.0	
Polypeptone Peptone	13.1	5.2	0.4	8.06	9.7	4.9	2.7	7.3	271	16.7	342	7340	44257	1.00	0.40	3.40	1.2	2.4	0.4	0.4	0.3	0.9	0.1	
Bacto Proteose Peptone	14.3	2.8	0.2	12.02	7.8	3.0	4.9	6.7	120	13.5	261	9123	29730	2.65	0.19	0.64	0.5	0.4	0.1	0.4	0.4	0.7	0.0	
BiTek Proteose Peptone	13.1	3.1	0.24	10.3	13.1	4.8	10.3	6.8	219	12.0	680	7390	44750	4.93	1.01	0.94	0.8	0.4	0.1	0.6	0.4	0.4	0.1	
Bacto Proteose Peptone No. 2	12.9	5.0	0.39	18.07	12.1	3.5	7.1	7.3	151	10.2	212	13313	47610	3.86	0.38	1.88	1.6	1.4	0.5	1.1	1.0	1.8	0.1	
Bacto Proteose Peptone No. 3	13.4	3.7	0.28	17.94	10.5	2.3	6.6	7.4	132	23.7	103	13160	38113	2.54	0.37	1.51	0.9	0.8	0.3	0.6	0.6	1.2	0.0	
BiTek Proteose Peptone No. 3	12.8	3.1	0.24	12.35	13.1	1.3	12.5	6.7	129	10.6	214	8682	50153	9.40	0.17	1.22	0.8	0.8	0.1	0.7	1.2	0.4	0.1	
Bacto Proteose Peptone No. 4	14.3	2.7	0.19	12.17	7.8	3.3	3.9	7.0	169	12.5	280	9109	35280	2.63	0.34	0.72	0.5	0.4	0.1	0.3	0.3	0.6	0.0	
Bacto Tryptose	13.3	4.5	0.34	10.56	8.8	3.2	3.2	7.3	191	34.2	110	9292	37740	1.61	0.23	2.05	1.2	1.9	0.4	0.5	0.4	1.3	0.0	

Legend

▣ Free amino acids ☐ Total amino acids

0.0 Below limit of detection

The data in this table represent the typical amounts of each component in each product and are not specifications. Multiple lots of each product were tested and the results in the table are the averaged values of each component.

* Partially destroyed during hydrolysis

** Utilizes an animal based enzyme in the digestion of the soy flour

For analytical method see Methods of Detection

	Glycine (% free)	Histidine (% free)	Isoleucine (% free)	Leucine (% free)	Lysine (% free)	Methionine (% free)	Phenylalanine (% free)	Proline (% free)	Serine (% free)	Threonine (% free)	Tryptophan (% free)	Tyrosine (% free)	Valine (% free)	Alanine (% total)	Arginine (% total)	Aspartic acid (% total)	Glutamic acid (% total)	Glycine (% total)	Histidine (% total)	Isoleucine (% total)	Leucine (% total)	Lysine (% total)	Methionine (% total)*	Phenylalanine (% total)	Proline (% total)	Serine (% total)*	Threonine (% total)	Tyrosine (% total)	Valine (% total)
	0.5	0.4	1.3	3.8	4.0	0.8	2.5	0.3	0.8	0.6	0.7	0.6	1.4	4.0	2.8	5.5	14.6	2.3	2.1	5.1	7.2	5.7	1.6	5.0	5.7	2.1	1.8	1.5	5.4
	1.0	0.1	0.2	0.4	0.6	0.3	0.2	0.4	0.3	0.2	0.2	0.3	0.2	7.1	4.2	2.4	6.4	8.2	1.4	1.3	2.8	2.5	0.7	1.5	7.2	0.3	0.4	0.8	2.0
	0.5	0.3	0.5	0.9	2.0	0.3	1.1	0.1	0.2	0.1	0.0	0.5	0.3	8.8	6.3	4.7	7.9	16.8	1.0	1.6	3.2	3.3	0.8	2.4	9.7	1.8	0.9	0.6	2.3
	0.2	0.1	0.3	1.6	0.8	0.5	1.3	0.1	0.3	0.2	0.3	0.8	0.3	4.3	2.6	4.2	7.4	3.4	1.2	2.3	4.6	4.0	1.0	2.7	4.7	0.8	0.9	2.2	2.9
	0.7	0.2	0.6	1.6	2.2	0.3	1.4	0.3	0.4	0.3	0.3	0.5	0.7	9.2	5.8	5.0	8.1	15.9	0.8	2.1	3.8	3.4	0.7	2.8	8.8	1.5	1.1	0.6	2.8
	0.5	0.4	1.1	3.9	3.6	1.0	2.4	0.3	0.7	0.7	0.6	0.7	1.3	4.1	3.3	6.1	12.6	3.0	2.1	3.8	6.2	6.2	1.9	3.6	5.4	2.1	1.9	1.6	4.7
	0.2	0.1	0.3	1.4	0.8	0.3	1.0	0.1	0.2	0.2	0.1	0.6	0.2	6.0	4.7	5.3	8.4	8.2	1.3	3.3	5.7	4.2	1.4	3.6	4.6	1.7	1.5	1.8	3.7
	0.4	0.1	0.4	1.4	0.9	0.6	1.1	0.1	0.2	0.1	0.1	0.5	0.4	7.0	4.4	3.9	6.3	7.3	0.8	2.0	4.2	3.4	1.0	2.3	6.3	0.3	0.7	1.2	2.8
	0.9	0.3	1.1	3.3	2.5	0.8	2.2	0.5	0.8	0.6	0.5	0.7	1.0	5.2	4.1	5.5	7.5	6.2	1.3	3.7	6.2	4.2	1.2	3.9	3.8	1.9	1.7	1.3	4.0
	0.4	0.1	0.6	2.3	1.5	0.6	1.5	0.3	0.5	0.4	0.3	0.8	0.5	5.2	4.3	5.1	8.0	6.5	1.3	3.2	5.6	4.2	1.3	3.5	3.8	1.6	1.5	1.6	3.5
	0.3	0.1	0.3	1.5	0.3	0.7	0.9	0.7	0.3	0.4	0.0	1.0	0.7	6.4	5.1	5.7	11.3	1.1	1.1	2.5	4.7	4.2	1.2	2.6	6.5	1.6	0.5	1.9	3.6
	0.2	0.1	0.3	1.2	0.7	0.5	0.9	0.1	0.2	0.2	0.2	0.5	0.2	6.5	4.6	4.4	6.5	5.9	1.1	2.2	4.3	4.0	1.1	2.3	5.0	0.4	0.8	1.6	2.9
	0.4	0.3	1.0	3.5	3.5	0.9	2.2	0.4	0.7	0.6	0.5	0.6	1.3	4.3	3.5	5.1	10.6	4.4	1.5	4.0	6.4	4.9	1.6	4.0	4.8	1.8	1.6	1.4	4.4

Typical analyses: Animal origin peptones

Casein and whey peptones

Gibco product	Total nitrogen (%)	Amino nitrogen (%)	AN/TN	Total carbohydrate (mg/g)	Ash (%)	Loss on drying (%)	NaCl (%)	pH (1% solution)	Calcium (µg/g)	Iron (µg/g)	Magnesium (µg/g)	Potassium (µg/g)	Sodium (µg/g)	Chloride (%)	Sulfate (%)	Phosphate (%)	Alanine (% free)	Arginine (% free)	Asparagine (% free)	Aspartic acid (% free)	Cystine (% free)	Glutamic acid (% free)	Glutamine (% free)	
Acidicase Peptone	8.5	6.2	0.73	0.29	36.8	5.3	32.3	6.8	229	4.9	36	383	140900	16.99	0.25	1.42	1.6	1.3	0.0	3.4	0.8	8.3	0.0	
Biosate Peptone	13.4	6.0	0.45	32.98	7.7	6.6	0.3	7.1	258	56.2	398	21320	17100	0.07	0.43	3.19	2.4	2.1	0.9	0.9	0.3	3.5	0.3	
Bacto Casamino Acids	10.8	9.4	0.87	0.0	18.3	4.8	12.1	6.4	59	1.3	143	4098	88090	6.74	0.55	2.56	3.0	2.4	0.0	0.7	0.1	15.1	0.0	
Bacto Casamino Acids, Technical	8.3	5.9	0.71	0.15	36.0	1.2	30.1	6.9	110	6.2	48	1361	145667	18.25	0.26	1.53	2.1	1.1	0.0	3.1	0.4	5.1	0.0	
Difco Casein Digest	13.2	7.0	0.53	1.44	10.0	5.0	0.0	3.7	163	2.8	49	1091	23923	0.26	0.1	0.57	1.6	2.9	1.6	1.1	2.4	3.6	0.1	
Bacto Casitone	13.5	5.0	0.37	3.54	6.4	2.0	0.0	7.0	111	23.5	213	3480	34090	0.1	0.4	2.48	0.9	2.6	0.5	0.2	0	0.9	0.0	
Bacto TC Lactalbumin Hydrolysate	13.0	6.3	0.48	21.01	7.2	4.6	0.3	7.0	1620	50.3	340	17200	14800	0.8	1.2	4.1	2.3	2.2	0.9	0.9	0.2	6.6	0.3	
Difco Tryptone	14.2	5.2	0.37	3.99	5.7	4.0	0.1	7.2	295	33.5	110	588	26600	0.09	0.18	2.54	0.8	2.5	0.5	0.3	0.0	1.1	0.4	
Bacto Tryptone	13.3	5.3	0.4	4.3	6.6	2.3	0.0	7.3	256	23.0	195	3257	33910	0.06	0.33	2.58	1.0	2.2	0.6	0.4	0.3	1.4	0.1	
BiTek Tryptone	13.1	5.6	0.43	8.42	5.8	5.0	0.0	7.1	387	7.3	100	620	26970	0.35	0.22	2.25	0.6	3.8	0.5	0.1	0.4	0.7	0.1	

Legend

☐ Free amino acids ☐ Total amino acids

0.0 Below limit of detection

The data in this table represent the typical amounts of each component in each product and are not specifications. Multiple lots of each product were tested and the results in the table are the averaged values of each component.

* Partially destroyed during hydrolysis

For analytical method see Methods of Detection

Glycine (% free)	Histidine (% free)	Isoleucine (% free)	Leucine (% free)	Lysine (% free)	Methionine (% free)	Phenylalanine (% free)	Proline (% free)	Serine (% free)	Threonine (% free)	Tryptophan (% free)	Tyrosine (% free)	Valine (% free)	Alanine (% total)	Arginine (% total)	Aspartic acid (% total)	Glutamic acid (% total)	Glycine (% total)	Histidine (% total)	Isoleucine (% total)	Leucine (% total)	Lysine (% total)	Methionine (% total)*	Phenylalanine (% total)	Proline (% total)	Serine (% total)*	Threonine (% total)	Tyrosine (% total)	Valine (% total)
0.8	0.8	1.6	3.9	4.4	0.9	2.5	3.3	2.1	0.9	0.0	1.0	1.8	2.1	1.9	3.9	11.6	1.0	1.6	4.0	6.3	4.6	1.4	3.5	5.3	2.5	1.4	1.4	4.4
0.6	0.6	1.6	4.7	3.5	1.0	2.9	0.5	1.0	0.8	0.7	0.5	1.9	4.2	2.9	5.9	16.1	2.2	2.0	5.8	7.7	5.9	1.9	5.5	6.2	2.2	1.9	1.4	6.1
1.4	0.2	3.1	4.6	2.1	1.4	3.4	7.5	0.4	0.5	0.0	0.4	4.7	3.0	2.5	2.4	15.9	1.4	0.8	4.0	5.0	5.2	1.4	3.6	8.0	2.1	1.5	0.4	5.6
0.8	0.5	1.2	2.7	4.0	0.9	1.4	2.9	2.1	0.9	0.0	1.5	1.6	4.4	1.7	3.4	8.4	1.1	1.1	2.7	4.6	4.6	1.2	1.9	5.7	1.6	0.5	1.6	3.4
0.5	1.3	2.5	6.8	5.4	2.3	3.5	1.1	1.7	2.0	7.2	3.4	3.1	2.7	5.1	6.0	16.8	1.7	2.2	3.9	7.8	6.7	2.7	4.0	7.4	4.2	2.2	3.6	5.5
0.2	0.4	1.1	4.7	4.5	1.1	2.7	0.3	0.8	0.5	0.8	0.5	1.3	3.4	2.8	5.5	16	1.7	1.9	5.9	7.9	5.9	2.2	5.5	7.1	2.1	1.9	1.6	6.3
1.3	0.5	2.1	3.5	2.5	1.6	0.8	0.5	1.5	1.3	0.6	0.8	2.4	4.7	2.5	6.5	8.7	2.7	1.1	3.6	4.9	8.4	2.5	2.3	1.1	4.2	1.4	0.9	3.7
0.2	0.4	1.0	5.3	4.4	1.1	2.4	0.1	0.6	0.7	0.8	0.4	1.6	3.1	3.5	7.4	20.2	1.9	2.6	5.4	9.1	8.2	2.7	4.5	10.2	4.7	3.9	1.9	6.7
0.2	0.5	1.3	4.8	5.5	1.0	3.0	0.2	0.7	0.7	0.8	0.5	1.7	3.2	5.0	5.2	15.1	1.7	1.9	5.5	7.5	6.2	2.1	5.2	6.6	2.2	1.8	1.3	5.9
0.1	0.6	1.1	4.2	5.4	0.7	2.8	0.1	0.7	0.7	0.8	0.4	1.5	5.0	2.6	3.9	9.8	1.4	1.6	3.8	6.0	5.9	1.4	3.4	7.3	0.3	0.8	1.2	4.6

Starter Paks

Starter Paks provide conveniently packaged 100 g samples of the peptones most commonly used in mammalian cell culture and microbial fermentation bioproduction processes. Our Starter Paks are tailored for specific applications, including the production of monoclonal antibodies, recombinant proteins, and vaccines.

Ultra-filtered peptones ideal for human health applications

Starter Pak No. 1* features a combination of yeast and soy-based peptones. Three of the products in this pack have been ultra-filtered to reduce endotoxin levels. The yeast products add a mixture of peptides, amino acids, carbohydrates (simple and complex), nucleosides and vitamins to any medium formulation. All of these products have been successfully used in human and animal health applications.

- **Difco TC Yeastolate UF**
  Bacto TC Yeastolate
  These peptones are ideal for CHO-based applications of biotherapeutic monoclonal antibodies and recombinant proteins.
- **Difco Yeast Extract UF**
  Bacto Yeast Extract, Technical
  These peptones support optimal growth of many microbial species for a variety of human and animal health vaccines.
- **Difco Phytone Supplement UF**
  This enzymatic digest of soy is a nutritious, excellent source of carbohydrates and is used in mammalian cell culture. This peptone works well alone and when blended with yeast-based peptones.

Animal origin–free and animal origin peptones best suited for vaccine production

Starter Pak No. 2* offers many essential nutrients needed for the production of human and animal vaccines.

- **Bacto Yeast Extract**
  This yeast has the highest level of carbohydrates of our yeast products and works well in a variety of human and animal health vaccines.
- **Phytone Peptone**
  Difco Soytone
  Both enzymatic digests of soy, these peptones also are a nutritious source of carbohydrates. These products work well in microbial fermentation processes as well as in mammalian cell culture processes such as CHO. Blending these soy peptones with yeast peptones has been shown to provide additional benefit to cultures.
- **Bacto Proteose Peptone No. 2**
  Bacto Proteose Peptone No 3
  These enzymatic digests of porcine protein provide nutrition for fastidious microorganisms. Bacto Proteose Peptone No. 3 can replace serum in many applications and helps increase monoclonal antibody and recombinant protein production in CHO cells.
- **Bacto Casamino Acids**
  This supplement has low salt and iron content, making it an excellent supplement for media formulations for which nitrogen requirements are minimal.

Animal origin–free peptones for animal and human vaccine production

Starter Pak No. 3* provides a variety of yeast and soy products, ideal for processes when an animal origin–free medium is preferred.

- **Bacto Yeast Extract**
  Gibco Yeast Extract
  These peptones contain a mixture of peptides, amino acids, carbohydrates, and vitamins to support optimal growth in microbial species, and are ideal for the production of vaccines.

- **Bacto TC Yeastolate**
  This peptone is ideal for CHO-based applications of biotherapeutic monoclonal antibodies and recombinant proteins, as well as vaccine applications

- **Phytone Peptone**
  Difco Soytone
  These soy-based supplements provide a nutritious source of carbohydrates for successful use in microbial fermentation. Blending these soy peptones with yeast peptones has been shown to provide additional benefit to cultures.

- **Bacto Malt Extract**
  A water-soluble portion of malted barley, this peptone also provides carbohydrates for a variety of microbial fermentation processes.

Ordering information

Product name	Cat. No.
Starter Pak No. 1	215366
Starter Pak No. 2	215367
Starter Pak No. 3	215368

Refer to the “Protocols and definition of methods” section for supplement titration and blending protocols to help quickly identify the right peptone supplementation for your process.

* Starter Paks are non-GMP and for evaluation use only.

Chemically defined supplements and feeds

Peptones have a long history of successful use as supplements in media systems for both mammalian and microbial applications. Animal origin–free (AOF) peptones and extracts mitigate the safety risk of using either serum or animal origin peptones; however, inherent in raw materials derived from a biological source, these AOF peptones and extracts are biological products with potential for variability [1]. In an effort to increase safety and reduce production and product variability, an increased emphasis has been placed on the use of AOF chemically defined (CD) supplements in bioprocess manufacturing.

CD supplements and feeds are formulations where each component can be linked directly back to a chemical with a defined CAS number. These formulations can be used in mammalian and bacterial applications [2]. These products should be used in conjunction with an optimized base medium in protocols that are similar to the optimization of peptone supplements. CD supplements and feeds have been developed to be used in batch and fed-batch processes to enhance recombinant protein production while, in some cases, improving product quality. Selected supplements can be tested on a variety of host cell lines and in various culture systems. Cell systems, defined by the combination of the host cell, recombinant protein expression system, basal medium, and bioprocess, are unique for each product. Therefore, any single supplement may not meet the needs of multiple cell lines or multiple processes. It is recommended that each new cell system should be optimized individually to achieve the goals of titer, viability, cell growth, and/or protein quality.

Note: While AOF-derived CD supplements can reduce the risk of animal origin sourced ingredients, as well as reduce lot-to-lot variability, CD supplements may not be as simple to use as a peptone. Peptones can bring nutritional and buffering capabilities to a cell system which may allow it greater flexibility during optimization. CD supplements and feeds are often coupled with CD basal media—the combination of the pair must account for all of the cellular requirements for nutritional, metabolic, and physical buffering needs.

References

1. Lee, Hae Woo, Andrew Christie, Jun Jay Liu, and Seongkyu Yoon. 2012. "Estimation of Raw Material Performance in Mammalian Cell Culture Using near Infrared Spectra Combined with Chemometrics Approaches." *Biotechnology Progress* 28.3:824-32.
2. Ma, Ningning, Joann Ellet, Centy Okediadi, Paul Hermes, Ellen Mccormick, and Susan Casnocha. 2009 "A Single Nutrient Feed Supports Both Chemically Defined NS0 and CHO Fed-batch Processes: Improved Productivity and Lactate Metabolism." *Biotechnology Progress* 25.5:1353-363.

Recharge Supplement without Glucose and L-Glutamine

Product description

Recharge Supplement is a chemically defined (CD) supplement designed to increase protein yields, while maintaining acceptable viability and cell growth. The supplement has been developed using several host cell lines and in various culture systems, including shaker flasks and bench-scale bioreactors. In order to increase versatility of the formulation, glucose and L-glutamine are not included in the final formulations. Therefore, these two components should be supplemented by the user and monitored as appropriate.

Potential applications

The Recharge Supplement was developed for use in CHO mammalian cell culture systems and can be used either as a media supplement or a culture system feed. However, user data have shown this CD supplement has been beneficial for non-CHO mammalian systems and microbial systems. Additionally, the Recharge Supplement has been successfully used as a replacement for yeast extract (Figure 1) [1,2]. Regardless of the cell system, the approach to optimizing the use of Recharge Supplement is similar [3]: 1) titrate Recharge Supplement in a small-scale platform to identify optimal concentrations, 2) optimize the feed strategy. Recharge Supplement can be used as a supplement to base media, in batch or in fed-batch processes. As with all chemically defined supplements and feeds, base media may need to be optimized to synergize with Recharge Supplement [4].

Physical characteristics

Recharge is a white to off-white, free-flowing powder.

Ordering information

Product name	Size	Cat. No.
Recharge Supplement without Glucose and L-Glutamine	100 g	670002
	1 kg	670003
	5 kg	670004

References

1. Oliver, Chaturvedi, Barbacci, Hunt, Dodson. 2011. "Development of Bio-Inspired, Chemically Defined Media Supplement for Cell Culture."
2. Chen, Merck. 2011. "Rapid Development of Chemically Defined Media and Feeds Through Replacement of Basal Hydrolysates." BioProcess International Annual Meeting, Long Beach, CA.
3. Chen. 2012. http://www.bioprocessintl.com/sponsored-content/
4. Recharge™ User Manual.

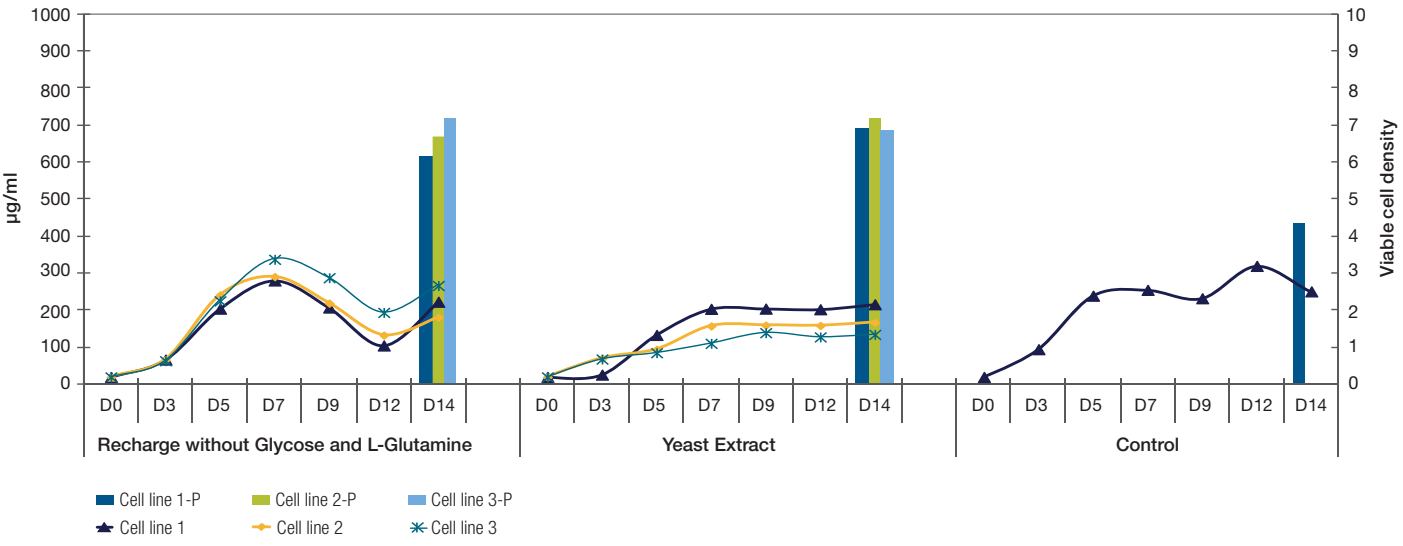


Figure 1. Performance of Recharge without Glucose and L-Glutamine compared to yeast extract and media control.

Resurge Chemically Defined Supplements CD1–5 without Glucose and L-Glutamine

Product description

Resurge™ supplements are a diverse set of fully chemically defined, animal origin–free and protein-free cell culture supplements, which help promote cell growth and boost protein production. Resurge supplements can be used in batch and fed-batch processes to enhance production in mammalian cells while maintaining product quality.

Resurge formulations compliment a variety of base media and were developed using several host cell lines and in various culture systems, including shaker flasks and bench-scale bioreactors.

Resurge supplements are available as individual products and as part of the Resurge™ CD Pak. The Resurge CD Pak contains one of each of the five individual Resurge supplements at 100 grams each:

- Resurge CD1 Supplement without Glucose and L-Glutamine
- Resurge CD2 Supplement without Glucose and L-Glutamine
- Resurge CD3 Supplement without Glucose and L-Glutamine
- Resurge CD4 Supplement without Glucose and L-Glutamine
- Resurge CD5 Supplement without Glucose and L-Glutamine

Potential applications

Resurge CD supplement formulations were developed for use in CHO mammalian culture systems and can be used either as a media supplement or a culture system feed (Figure 2). User data have shown that these CD supplements have been beneficial for non-CHO mammalian systems and microbial systems. As with all chemically defined supplements and feeds, base media may need to be optimized to synergize with Resurge CD supplement. Regardless of the cell system, Resurge CD supplement should initially be evaluated on a small-scale platform to identify optimal concentrations, followed by optimization of the feed strategy. Resurge CD supplement can be used as a supplement to base media, in batch or fed-batch processes. These products have been shown to be scalable to bioreactor systems.

Physical characteristics

Resurge CD1 is a free-flowing, beige homogeneous powder.

Resurge CD2 is a free-flowing, beige homogeneous powder.

Resurge CD3 is a free-flowing, white to off-white powder.

Resurge CD4 is a free-flowing, white to off-white homogeneous powder.

Resurge CD5 is a free-flowing, white to off-white homogeneous powder.

Ordering information

Product name	Size	Cat. No.
Resurge CD1	100 g	670011
	1 kg	670012
	5 kg	670013
Resurge CD2	100 g	670015
	1 kg	670016
	5 kg	670017
Resurge CD3	100 g	670018
	1 kg	670019
	5 kg	670020
Resurge CD4	100 g	670021
	1 kg	670022
	5 kg	670023
Resurge CD5	100 g	670024
	1 kg	670025
	5 kg	670026
Resurge CD Pak	100 g x 5	670030

Usage instructions are provided in the “Protocols and definition of methods” section of this guide as a starting point for an evaluation.

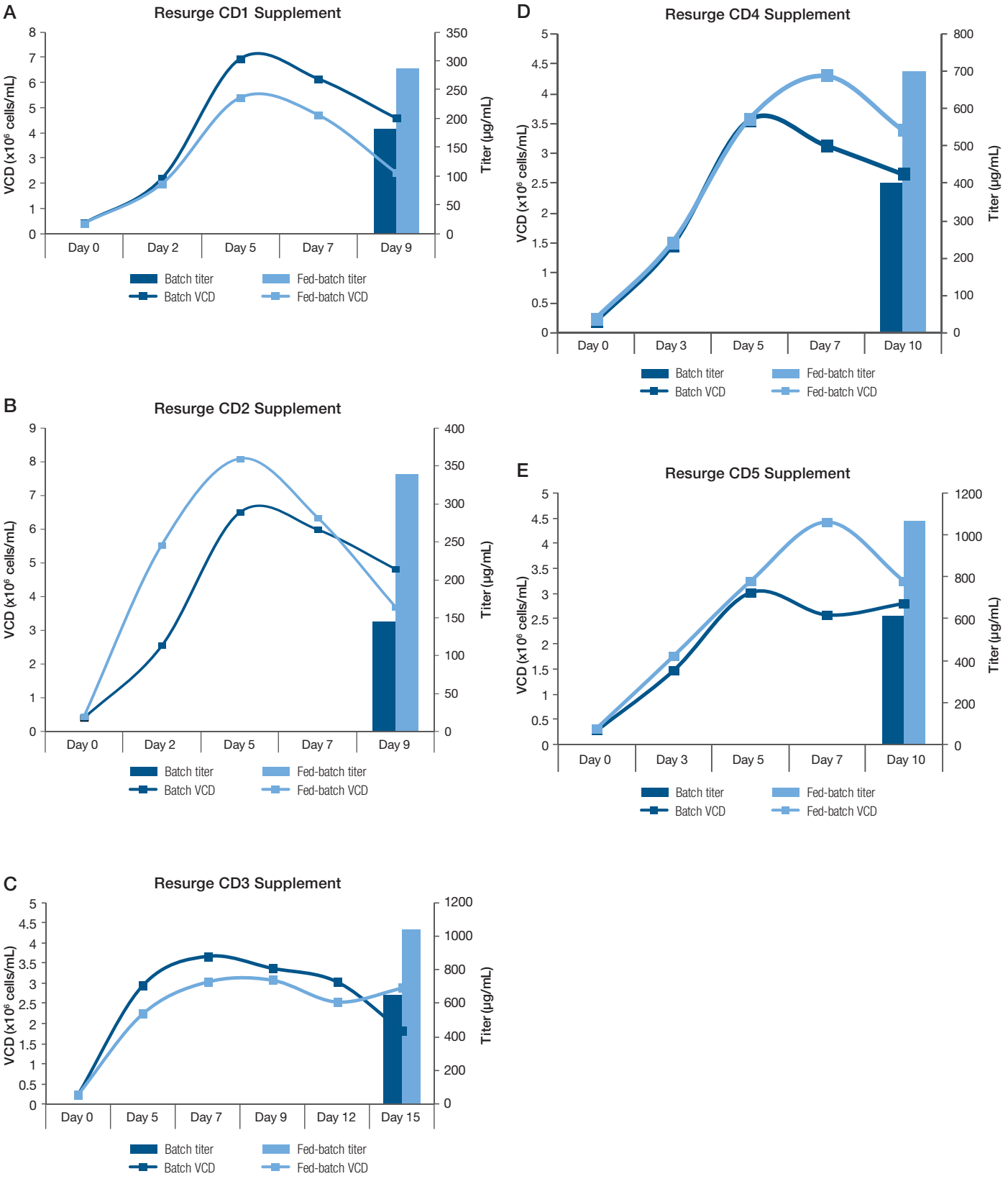


Figure 2. Performance of Resurge CD1 through CD5, batch vs. fed-batch processes.

OneFeed Supplement

Product description

Gibco™ OneFeed™ Supplement is a versatile chemically defined, animal origin–free, protein-free feed that can be used with a variety of media to enhance protein production. This high-performance feed has been developed using several different cell lines in various culture systems with a variety of commercially available media. OneFeed Supplement has a balanced nutritional profile that provides key components to support high growth and production throughout the culture life. OneFeed Supplement was co-developed with Celonic.

Application

OneFeed Supplement can be used as a feed supplement to enhance growth and production of a variety of CHO lines. As shown in Figure 3 below, OneFeed Supplement improves cell performance across media and cell lines. In these two studies, OneFeed Supplement was added to each culture using the protocol outlined in the "Protocols and definition of methods" section, and the result for each line was increased growth and production. Usage instructions are provided in the "Protocols and definition of methods" section of this guide as a starting point for an evaluation. If the provided method is not suitable, this flexible feed can be added to existing processes.

Physical characteristics

OneFeed Supplement is a homogeneous, free-flowing, off-white to light pink powder.

Ordering information

Product name	Size	Cat. No.
OneFeed Supplement	2 L powder	670110
	10 L powder	670109
	50 L powder	670108

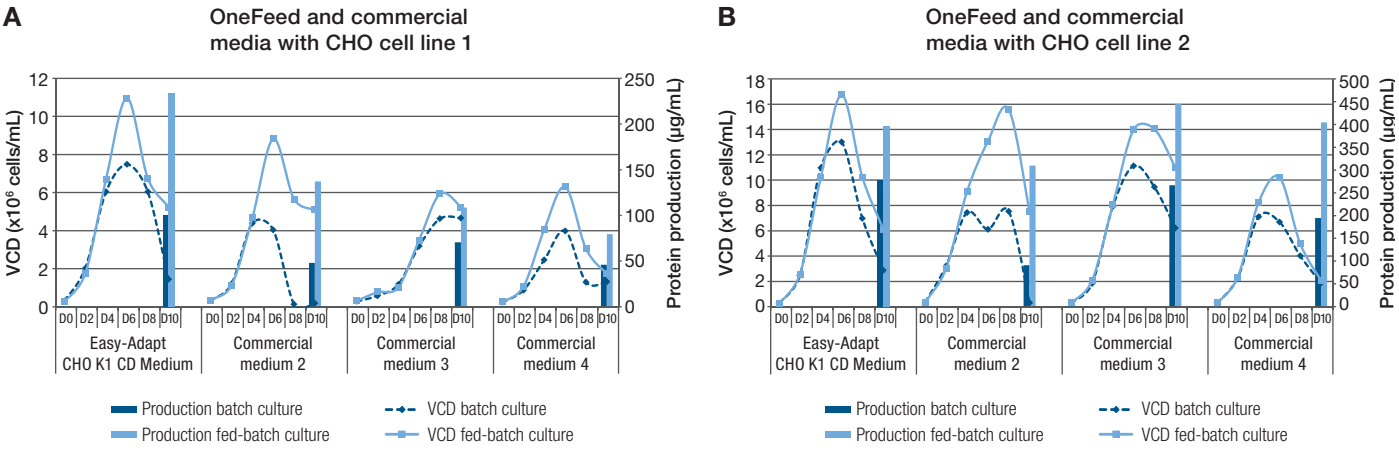


Figure 3. OneFeed Supplement with commercial media: Growth and production evaluation of CHO cell line 1 (graph A) and CHO cell line 2 (graph B) in commercial media in batch culture and in fed-batch culture with OneFeed Supplement in shake flasks.

Microbial and vaccine media

We offer multiple peptone-based media products used for microbial fermentation in the human and animal vaccine markets worldwide. These enriched complete media formulations consist of blends of peptones and can provide nutrition for enhancing growth and vaccine production for fastidious organisms, such as mycoplasmas, streptococci, pneumococci, and meningococci. Additionally, media formulations such as Tryptose Phosphate Broth, have been used for supplementing cell culture media for enhancing vaccine production.

Bacto Tryptose Phosphate Broth

Product description

Bacto Tryptose Phosphate Broth was designed as an alternative to classical animal-based media for the maintenance and propagation of *Escherichia coli* strains in molecular genetics procedures. It is manufactured from animal origin–free ingredients in order to minimize the risk of using culture media containing animal materials from TSE/BSE-relevant species.

Bacto Tryptose Phosphate Broth is an infusion-free buffered medium recommended for the cultivation of fastidious pathogenic microorganisms such as streptococci, *Neisseria*, and *Listeria* and for use in various cell culture applications. In the medium, peptones provide carbon and nitrogen; glucose is the primary source and sodium chloride maintains osmotic balance. Buffering capacity is provided by disodium phosphate.

Applications

Bacto Tryptose Phosphate Broth is a nutrient-rich formulation designed to out-perform classical animal-based molecular genetics media formulations.

Bacto Tryptose Phosphate Broth can be used in procedures for the serodiagnosis of *Listeria monocytogenes* and in tissue culture procedures [1], where the peptone content is considered to be a stimulating factor for cells. Bacto Tryptose Phosphate Broth is often used as a supplement at 5–10% (v/v) to serum containing medium for mammalian cell cultures, such as BHK cells, and for end application of viral vaccine production, such as foot and mouth disease (FMDV).[2] Media containing Bacto Tryptose Phosphate Broth has also been used for growing various mammalian cells for studies on viruses such as rabies using BHK cells [3], for propagation and studies of influenza virus in MDCK cells [4], and for growth of transgenic VERO and BHK-21 cell lines for propagation of dengue virus [5].

It has also been used for growth of a primary chicken kidney cell line in serum-containing medium for studies on infectivity of *Salmonella* species [6] and for propagation of a VERO cell line for studying infection by an intracellular parasite *Rickettsia* species that causes typhus in humans [7]. Bacto Tryptose Phosphate Broth, in combination with Difco TC Yeastolate UF, has been used as a part of serum-free media for production of recombinant proteins in Sf9 insect cell lines [8].

Formula

Approximate formula* per liter	
Tryptose	20.0 g
Dextrose	2.0 g
Sodium chloride	5.0 g
Disodium phosphate	2.5 g

* Adjusted and/or supplemented as required to meet performance criteria.

Suspend 29.5 g of the Bacto Tryptose Phosphate Broth in 1 L of purified water. If a medium containing 0.1–0.2% agar is desired, add 1–2 g of agar; heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Autoclave at 121°C for 15 minutes. Test samples of the finished product for performance using stable, typical control cultures.

Physical characteristics

Bacto Tryptose Phosphate Broth is a beige free-flowing homogeneous powder.

Ordering information

Product name	Size	Cat. No.
Bacto Tryptose Phosphate Broth	10 kg	260200

References

1. Ginsberg, Gold and Jordan. 1955. Proc. Soc. Exp. Biol. Med. 89:66.
2. Jerome Polatnick and Howard L. Bachrach. 1964. Production and Purification of Milligram Amounts of Foot-and-Mouth Disease Virus From Baby Hamster Kidney Cell Cultures, Applied Microbiology. 12:368-373.
3. Tomomi Nakahara, Harufusa Toriumi, Takashi Irie, Takuo Takahashi, Satoshi Ameyama, Masami Mizukoshi, and Akihiko Kawai. 2003. Characterization of a Slow-Migrating Component of the Rabies Virus Matrix Protein Strongly Associated with the Viral Glycoprotein Microbiol. Immunol., 47(12):977–988.
4. Yamamoto Goshima and K Maeno 1989. Persistent Infection of MDCK Cells by Influenza C Virus:Initiation and Characterization. J. gen. Virol. 70:3481-348.
5. Supranee Phanthanawiboon, Atchareeya A-nuegoonpipat, Narawan Panngarm,Kriengsak Limkittikul, Kazuyoshi Ikuta, Surapee Anantapreecha, Takeshi Kurosu. 2014. Isolation and propagation of Dengue virus in VERO and BHK-21 cells expressing human DC-SIGN stably, Journal of Virological Methods. 209:55–61.
6. Pete Kaiser, Lisa Rothwell, Edouard E. Galyov, Paul A. Barrow, Joan Burnside and Paul Wigley. 2000. Differential cytokine expression in avian cells in response to invasion by *Salmonella typhimurium*, *Salmonella enteritidis* and *Salmonella gallinarum* Microbiology, 146:3217–3226.
7. Paul F. Policastro,* Marius G. Peacock and Ted Hackstadt. 1996. Improved Plaque Assays for Rickettsia prowazekii in VERO76 Cells, Journal of Clinical Microbiology. 1944–1948.
8. S. Reuveny, Y. J. Kim, C. W. Kemp, and J. Shiloach. 1993. Communications to the Editor Production of Recombinant Proteins in High-Density Insect Cell Cultures, Biotechnology and Bioengineering. 42:235-239.

Difco PPLO Broth

Product description

Difco PPLO Broth is a complete media used in the cultivation of microorganisms from the class Mollicutes. *Mycoplasma* was first identified from a case of pleuropneumonia in a cow [1] and the causative organism was designated "pleuropneumonia-like organism," or PPLO [1]. Meat digests, peptones, beef extract, and yeast extract provide the nitrogen, vitamins, amino acids, and carbon for optimal growth of *Mycoplasma* species. Osmotic balance, which is critical for mycoplasmas as they lack a cell wall, is maintained using sodium chloride in the formulation. *Mycoplasma* species are fastidious and require supplementation of serum, such as horse serum to the broth media, which supplies cholesterol, a growth stimulant [2].

Application

PPLO (*Mycoplasma*) agars and broths, when supplemented with nutritive enrichments, are used for isolating and cultivating *Mycoplasma*. PPLO (*Mycoplasma*) Agar was described by Morton, Smith, and Leberman [3]. It was used in a study of the growth requirements of *Mycoplasma* [4] along with the identification and cultivation of this organism [5-7].

Difco PPLO Broth is used in animal health applications for the propagation of *Mycoplasma* species such as *Mycoplasma mycoides*, *Mycoplasma gallisepticum*, and others for vaccine production [8-10].

Formula

Approximate formula* per liter	
Beef heart, infusion from 50 g	6.0 g
Peptone	10.0 g
Sodium chloride	5.0 g

* Adjusted and/or supplemented as required to meet performance criteria.

Dissolve 21 g of the powder in 700 mL of purified water. Mix thoroughly.

Autoclave at 121°C for 15 minutes. Cool medium to 50–60°C. Aseptically add 300 mL mycoplasma supplement, such as horse serum, as appropriate to the medium. Mix well. Add selective agents if desired (i.e., thallium acetate or penicillin). Test samples of the finished product for performance using stable, typical control cultures. Solution pH at 25°C: 7.8 ± 0.2

Physical characteristics

Dehydrated appearance: Light beige, free-flowing, homogeneous.

Ordering information

Product name	Size	Cat. No.
Difco PPLO Broth	10 kg	255410

References

1. Baron, Peterson, and Finegold. 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc. St. Louis, Mo.
2. Waites and Taylor-Robinson. 1999. In Murray, Baron, Pfaller, Tenover, and Yolken (ed.). Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
3. Morton, Smith, and Leberman. 1951. Am. *J. Syphilis Gonorrh.* 35:361.
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5. Chanock, James, Fox, Turner, Mufso, and Hayflick. 1962. *Soc. Exp. Biol. Med.* 110:884.
6. Craven, Wenzel, Calhoun, Hendley, Hamory, and Gwaltney. 1976. *J. Clin. Microbiol.* 4:225.
7. Gregory and Cundy. 1970. *Appl. Microbiol.* 19:268.
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9. Charles H. Domermuth, Vaccination of Chickens with *Mycoplasma gallisepticum* Avian Diseases, Vol. 6, No. 4 (Nov. 1962), pp. 412-419.
10. Kazama, Yagihashi, Nunoya. 1986. *Microbiol. Immunol.* Vol. 30 (9), 923-929.

Difco Malt Extract Broth

Product description

Difco Malt Extract Broth is a complete microbiological media for the cultivation of yeasts and molds. Difco Malt Extract Broth contains malt extract, which provides the carbon, nitrogen, and nutrient sources required for growth of microorganisms. The broth also has a high concentration of carbohydrates, such as maltose and dextrose, as an energy source required for fermentation by yeasts and molds. Yeast extract provides additional nitrogen, carbon, vitamins, and cofactors required for growth. The acidic pH of Difco Malt Extract Broth promotes optimal growth of yeasts and molds while restricting bacterial growth.

Application

A culture medium prepared from malt extract that was a satisfactory replacement for wort was described by Reddish [1]. Thom and Church [2] further used malt extract as a base for complete media preparation for the growth of *Aspergillus*. Malt Extract Broth is recommended for yeasts and molds in the U.S. Food and Drug Administration's Bacteriological Analytical Manual [3].

Formula

Approximate formula* per liter	
Malt Extract	6.0 g
Maltose, Technical	1.8 g
Dextrose	6.0 g
Yeast Extract	1.2 g

* Adjusted and/or supplemented as required to meet performance criteria.

Dissolve 15 g of the powder in 1 L of purified water. Autoclave at 121°C for 15 minutes. Test samples of the finished product for performance using stable, typical control cultures. Solution pH at 25°C: 4.7 ± 0.2

Physical characteristics

Light beige to beige, free-flowing, homogeneous.

Ordering information

Product name	Size	Cat. No.
Difco Malt Extract Broth	10 kg	214912

References

1. Reddish. 1919. Abstr. Bacteriol. 3:6.
2. Thom and Church. 1926. The aspergilli. Williams & Wilkins, Baltimore, Md.
3. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.

Bacto Brain Heart Infusion

Difco Brain Heart Infusion, without Dextrose

Bacto Brain Heart Infusion, Porcine

Product description

Bacto Brain Heart Infusion is a microbiological culture medium used for cultivating fastidious microorganisms, including streptococci, pneumococci, and meningococci. In 1919, Rosenow [1] devised an excellent medium for culturing streptococci by supplementing dextrose broth with brain tissue. Hayden [2] revised Rosenow's procedure by adding crushed marble to the medium and reported favorable growth of dental pathogens. Brain Heart Infusion (BHI) is a modification of the media described by Rosenow [1] and Hayden [2] in which infusion from calf brains has replaced the brain tissue, and disodium phosphate has replaced the calcium carbonate.

Infusion from beef heart, calf brains, and Proteose Peptone provide nitrogen, carbon, sulfur and vitamins in Brain Heart Infusion media. Dextrose is a carbon energy source to facilitate organism growth, Sodium chloride maintains osmotic balance in the medium, and disodium phosphate is a buffering agent.

Difco Brain Heart Infusion without Dextrose is the same formulation as Bacto BHI with no dextrose added. It is a basal medium that is generally used with carbohydrates for fermentation studies.

Bacto Brain Heart Infusion, Porcine was developed as an alternative to the classical Brain Heart Infusion formula, and replaces calf brains and beef heart with pork brains and heart. Brain Heart Infusion, Porcine was formulated with no bovine components to minimize bovine spongiform encephalopathy (BSE) risk.

Infusion from pork brains, infusion from pork heart, and Proteose Peptone No. 2 provide nitrogen, carbon, sulfur, and vitamins in Brain Heart Infusion, Porcine. Dextrose is a carbon energy source to facilitate organism growth, Sodium chloride maintains osmotic balance in the medium, and disodium phosphate is a buffering agent.

Applications

Brain Heart Infusion media are specified in several standard methods references for food testing [3-5]. *Standard Methods for the Examination of Water and Wastewater* recommends Brain Heart Infusion media in tests for the verification of fecal streptococci [6]. Brain Heart Infusion is listed by the National Committee for Clinical Laboratory Standards (NCCLS) as a medium for use in the preparation of microdilution trays for antimicrobial susceptibility testing by the broth microdilution procedure [7].

BHI broth has been used in various microbiological studies. BHI is recommended for the growth of most ATCC™ strains of *Pasteurella multocida* and has been cited in many studies on *P. multocida* fowl cholera vaccines. Duffy et al. [8] used BHI as a fermentation medium for pH studies on *Escherichia coli* O157:H7. Tan et al. [9] utilized BHI to grow *Fusobacterium necrophorum* for leukotoxin production studies. Likewise, Van Tassell et al. [10] made enterotoxin preparations by growing *Bacteroides fragilis* in BHI. BHI, Porcine was developed for pharmaceutical and vaccine production and can replace the traditional BHI depending on organism and production application.

Formula—Bacto Brain Heart Infusion

Approximate formula* per liter	
Calf brains, infusion from 200 g	7.7 g
Beef heart, infusion from 250 g	9.8 g
Bacto Proteose Peptone	10.0 g
Dextrose	2.0 g
Sodium chloride	5.0 g
Disodium phosphate	2.5 g
Solution pH at 25°C: 7.4 ± 0.2	

* Adjusted and/or supplemented as required to meet performance criteria.

Formula—Bacto Brain Heart Infusion, Porcine

Approximate formula* per liter	
Pork brains, infusion from 200 g	7.7 g
Pork heart, infusion from 250 g	9.8 g
Bacto Proteose Peptone No. 2	10.0 g
Dextrose	2.0 g
Sodium chloride	5.0 g
Disodium phosphate	2.5 g
Solution pH at 25°C: 7.4 ± 0.2	

* Adjusted and/or supplemented as required to meet performance criteria.

Physical characteristics

Bacto Brain Heart Infusion is a light tan, free-flowing, homogeneous powder.

Bacto Brain Heart Infusion, Porcine is a light tan, free-flowing, homogeneous powder.

Difco Brain Heart Infusion, Without Dextrose is a light tan, free-flowing, homogeneous powder.

Ordering information

Product name	Size	Cat. No.
Bacto Brain Heart Infusion	10 kg	237300
Bacto Brain Heart Infusion, Porcine	10 kg	256110
Difco Brain Heart Infusion, Without Dextrose	10 kg	250220

References

1. Rosenow. 1919. Studies on elective localization. *J. Dent. Res.* 1:205-249.
2. Hayden. 1923. Elective localization in the eye of bacteria from infected teeth. *Arch Intern Med.* 32:828-849.
3. Horwitz (ed.). 2000. Official methods of analysis of AOAC International, 17th ed. AOAC International, Gaithersburg, MD.
4. Food and Drug Administration. 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
5. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, DC.
6. Clesceri, Greenberg and Eaton (ed.). 1998. Membrane filter techniques, 9-72-74. Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association, Washington, DC.
7. National Committee for Clinical Laboratory Standards. 1997. Methods for antimicrobial susceptibility testing of anaerobic bacteria, 4th ed. Approved standard M11-A4. National Committee for Clinical Laboratory Standards, Wayne, PA.
8. Duffy, Riordan, Sheridan, Call, Whiting, Blair and McDowell. 2000. Effect of pH on survival, thermotolerance, and verotoxin production of *Escherichia coli* O157:H7 during simulated fermentation and storage. *J Food Prot.* 63:12-18.
9. Tan, Nagaraja and Chengappa. 1992. Factors affecting leukotoxin activity of Fusobacterium necrophorum. *Vet Microbiol.* 32:15-28.
10. Van Tassell, Lyerly and Wilkins. 1992. Purification and characterization of an enterotoxin from *Bacteroides fragilis. Infect Immun.* 60:1343-1350.

Protocols and definition of methods

Supplement titration and blending protocols

Since every supplement and feed is different, and each cell line has unique nutritional requirements, it is critical to evaluate a wide range of peptones or CD supplements. Even multiple peptones produced using the same protein source should be considered. To quickly identify supplements that improve culture performance, a titration of each peptone or CD supplement should be performed to identify both products and concentrations that should be further evaluated. Synergistic effects have been observed when blending peptones or CD supplements, so additional mixture designs are also recommended. When performing the screening studies, it is important to add any cell-specific supplementation to the media. The following titration and blending protocols can be used as provided, or as a starting point for designing custom screening studies.

Peptone procedure

Mammalian cell culture application

Reconstitution Instructions

Peptone powders are recommended to be hydrated at 100 g/L for mammalian cell culture using the following instructions:

- Weigh 10 grams of peptone powder.
- Fill a clean 250 mL beaker with approximately 90 mL room temperature water for injection (WFI) or equivalent. Add the peptone to the beaker and mix until completely dissolved.
- Once completely dissolved, bring volume to 100 mL with WFI or equivalent.
- Sterilize the solution by filtration through a 0.2 µm filter membrane or by autoclaving. Store solution at 2–8°C.

Recommended testing procedure

Titration study—batch culture in shake flasks

- **Step 1:** Prepare shake flasks by adding an appropriate volume of reconstituted peptone (100 g/L stock solution) and basal medium to the final concentrations shown in Table 1. In addition to the components in Table 1, cultures should be supplemented with glucose, L-glutamine, Pluronic™ F 68 (Kolliphor™ P 188), and any other cell line–specific supplements as required.
- **Step 2:** Prepare seeding cell culture according to standard protocols. If cells are being cultured in a peptone-containing medium, wash cells once in sterile PBS and pellet by centrifugation. Prepare seeding cell suspension by resuspending the cell pellet in base medium.
- **Step 3:** Inoculate shake flasks with standard seeding density.
- **Step 4:** On days 0, 3, 5, 7, 10, and the last day of cell culture, determine viable cell density and percent viability.
- **Step 5:** Adjust glucose and glutamine levels as appropriate for the base medium throughout the experiment.
- **Step 6:** Determine protein titer on various days of culture, including the last day, to determine cumulative protein production.

Table 1. Titration study experimental set up (mammalian cell culture).

Note: This table provides guidance for titration of one peptone; similar setups can be used for all peptones; add appropriate positive control if needed.

Condition	Gibco peptone concentration (g/L)	Volume of base medium (mL)	Volume of Gibco peptone stock (100 g/L) (mL)
Concentration-1	1	49.50	0.5
Concentration-2	3	48.50	1.0
Concentration-3	6	47.00	3.0
Concentration-4	9	45.50	4.5
Medium only (negative control)	0	50.00	0

Mixture study—batch culture in shake flasks

The three top-performing peptones selected from the titration studies can be mixed as per Table 2 to further optimize performance. Table 3 provides instructions for the experimental set up using a 100 g/L stock solution.

Table 2. Mixture study (mammalian cell culture).

Mixture	Gibco peptone 1 (g/L)	Gibco peptone 2 (g/L)	Gibco peptone 3 (g/L)	Total peptone (g/L)
Mixture 1	2.0	0.5	0.5	3.0
Mixture 2	2.0	2.0	2.0	6.0
Mixture 3	0.5	2.0	0.5	3.0
Mixture 4	0.5	0.5	2.0	3.0
Mixture 5	0.5	0.5	0.5	1.5

Table 3. Mixture study experimental setup (mammalian cell culture)

Shake flask	Gibco peptone 1 stock (g/L)	Gibco peptone 2 stock (g/L)	Gibco peptone 3 stock (g/L)	Volume of base medium (mL)
Mixture 1	1	0.25	0.25	48.50
Mixture 2	1	1	1	47.00
Mixture 3	0.25	1	0.25	49.50
Mixture 4	0.25	0.25	1	45.50
Mixture 5	0.25	0.25	0.25	49.25
Control	0	0	0	50

Microbial cell culture application

Reconstitution instructions

Peptone powders are recommended to be hydrated at 30 g/L for microbial cell culture using the following instructions:

- Weigh 6 grams of the peptone powder.
- Fill a clean 500 mL beaker with approximately 180 mL of room temperature WFI, deionized water (DI), or equivalent purified water.
- Add the peptone to the beaker and mix until completely dissolved.
- Once completely dissolved, bring the final volume to 100% with room temperature WFI, deionized water (DI), or equivalent purified water.
- Measure pH at room temperature and adjust to between 6.5 and 7.4. Avoid excessive pH adjustments. Sterilize the solution by autoclaving or filtration using a 0.2 µm filter membrane.
- Store solution at 2–8°C.

Recommended testing procedure

Titration study in shake tubes or flasks

A peptone screen should be designed so each peptone is evaluated at a variety of concentrations. Typical working concentrations for peptones range from 5 g/L to 30 g/L and are dependent on the microorganism's need. Table 4 outlines a recommended peptone titration experimental study. In addition to the components in Table 4, additional supplementation may be required to support microorganism growth, such as buffer salts like phosphates or a base medium like M9 Minimal Salts. Further supplementation with carbohydrates, like glucose; growth factors, like serum or blood; mineral salts, like magnesium, calcium, or iron; and any other microbial specific supplements can be added as required for peptone optimization.

Mixture study in shake tubes or flasks

Blends of peptones should also be considered because synergistic effects can be observed in some processes when multiple peptones are used. The top two performing peptones from the titration study can be blended as outlined in Tables 5 and 6. Selection of the peptone or blend of peptones should be based on both the proliferation and production data.

Table 4. Titration study (microbial cell culture).

Concentration	Gibco peptone (g/L)	Volume buffer salts or purified water	Volume of Gibco peptone stock at 30 g/L	Total volume (mL)
Concentration 1	30	0.0	50.0	50
Concentration 2	20	16.7	33.3	50
Concentration 3	10	33.3	16.7	50
Concentration 4	5	41.7	8.3	50

Table 5. Mixture study (microbial cell culture).

Mixture	First Gibco peptone concentration (g/L)	Second Gibco peptone concentration (g/L)	Total Gibco peptone concentration (g/L)
Mixture 1	15	15	30
Mixture 2	12	8	20
Mixture 3	10	10	20
Mixture 4	8	12	20
Mixture 5	5	5	10

Table 6. Mixture experimental setup (microbial cell culture).

Mixture	Volume of first AB peptone stock in buffer salts or purified water (mL)	Volume of second AB peptone stock in buffer salts or purified water (mL)	Volume of buffer salts or purified water (mL)	Total volume (mL)
Mixture 1	20.0	13.3	16.7	50
Mixture 2	16.7	16.7	16.7	50
Mixture 3	13.3	20.0	16.7	50
Mixture 4	8.3	8.3	33.3	50
Mixture 5	25.0	25.0	0.0	50

CD supplement procedure

Resurge CD supplements are supplied as powders and should be hydrated at 40 g/L using the following instructions:

Step 1: Weigh 40 grams of CD supplement powder.

Step 2: Fill a clean 1 L beaker with approximately 900 mL of room temperature water for injection (WFI) or equivalent.

Step 3: Add the powder to the beaker and mix for a minimum of 30 minutes or until completely dissolved.

Step 4: Bring the volume to 1 L with WFI or equivalent.

Step 5: Filter-sterilize the concentrated solution through a 0.2 µm filter membrane and use as needed.

Step 6: Store the solution at 2–8°C and protect from light.

More concentrated stock solutions of Resurge CD supplements can be prepared using the following instructions for making a 100 g/L stock solution.

Step 1: Weigh 100 g of CD supplement powder.

Step 2: Fill a clean 1 L beaker with approximately 700 mL of room temperature water for injection (WFI) or equivalent.

Step 3: Add the powder to the beaker and mix until all the powder is completely dissolved.

Step 4: Adjust the pH to between 9.0 and 10.0 using 5 N or 6 N NaOH.

Step 5: Mix for a minimum of 30 minutes.

Step 6: Adjust the pH to 8.0 ± 0.2 or the desired pH using 5 N or 6 N HCl.

Step 7: Bring the volume to 1 L with WFI or equivalent.

Step 8: Mix for a minimum of 10 minutes.

Step 9: Filter-sterilize the concentrated solution through a 0.2 µm filter membrane and use as needed.

Step 10: Store the solution at 2–8°C and protect from light.

Testing procedure

For a batch culture process, we recommend an initial titration when using each Resurge CD supplement. For a batch culture, Resurge CD supplement(s) can be used at a final supplemental concentration of 1 g/L, 3 g/L, or 6 g/L (in the cell culture vessel) on day 0 or day 2 of the culture. Glucose and glutamine levels should be maintained per cell line requirements.

For a fed-batch process, Resurge CD supplements can be fed to a final supplemental concentration of 2–6 g/L (in the cell culture vessel) per day for multiple days, starting on day 0 or day 2, to mid-growth phase. The culture should be started at a reduced volume to accommodate the anticipated feeding volume. Glucose and glutamine levels should be maintained per cell line requirements.

Fed-batch process	
Day 0 or day 2 to mid-growth phase	2.0–6.0 g/L

Batch culture process	
Day 0	1.0 g/L
	3.0 g/L
	6.0 g/L

Resurge CD supplement troubleshooting

For problems encountered when using Resurge CD supplements, refer to the quick troubleshooting guide below.

Problem	Cause	Solution
Resurge CD supplements are either not dissolving or taking an excessively long time to completely dissolve	Low vortex due to slow mix speed or wrong reconstitution vessel	Use a beaker to reconstitute small amounts of Resurge CD supplements Increase the mixing speed to maximize the vortex
	The temperature of the water or room is unusually low	Reconstitute with room temperature (~25°C) water in an environment controlled at ~25°C
Medium is cloudy or a precipitate is observed following addition of Resurge CD supplements	Possible reaction between components resulting in precipitation	Perform a compatibility test to identify the correct concentration of Resurge supplement to use
	Concentration of some components above solubility limits	Contact Technical Services for troubleshooting unwanted chemical reactions
	Possible contamination	Identify the contaminant and root cause of contamination Retest using aseptic procedures
Cell culture performance is inadequate	High/low levels of Resurge supplements	Perform a Resurge titration study to determine the concentration range for optimal performance
	High osmolality when Resurge supplement added to basal medium	Perform a compatibility test to identify the optimum concentration of Resurge supplement to use for your application Reduce other supplementation levels
	Over-supplementation if using other feed additives (amino acids, hydrolysates, etc.) together with Resurge supplements	Try an alternate feeding plan with a reduced amount of other feeds (amino acids, hydrolysates, etc.)
	Resurge supplement not added on day 0 of culture	Try adding Resurge supplement on day 0 of culture. In some instances, this process change has improved performance

OneFeed Supplement usage instructions

OneFeed Supplement is recommended for use in mammalian cell culture to enhance growth and/or protein production in fed-batch processes. OneFeed Supplement is chemically defined (CD) and animal origin–free (AOF). OneFeed Supplement does not contain L-glutamine or hypoxanthine and thymidine (HT).

OneFeed Supplement 1 L hydration instructions

To prepare 1 L, add 900 mL of WFI-quality water or equivalent to an appropriate mixing vessel. Weigh approximately 40.3 g/L of OneFeed Supplement powder and add it to the mixing vessel. Mix for a minimum of 60 minutes. Adjust the pH to approximately 8.5 with 5 N or 6 N NaOH. Mix for a minimum of 10 minutes. Adjust the pH to 7.0 ± 0.5 with 5 N or 6 N HCl. Adjust to the final volume with WFI-quality water or equivalent. Mix for a minimum of 10 minutes. Filter-sterilize the solution using a 0.2 µm filter and store at 2–8°C until use. Protect from light.

Cat. No. 670110—OneFeed Supplement 2 L hydration instructions

To prepare 2 L, add 1.8 L of WFI-quality water or equivalent to an appropriate mixing vessel. Add the entire bottle contents to the vessel and mix for a minimum of 60 minutes. Adjust the pH to approximately 8.5 with 5 N or 6 N NaOH. Mix for a minimum of 10 minutes. Adjust the pH to 7.0 ± 0.5 with 5 N or 6 N HCl. Adjust to the final volume with WFI-quality water or equivalent. Mix for a minimum of 10 minutes. Filter-sterilize the solution using a 0.2 µm filter and store at 2–8°C until use. Protect from light.

Cat. No. 670109—OneFeed Supplement 10 L hydration instructions

To prepare 10 L, add 9 L of WFI-quality water or equivalent to an appropriate mixing vessel. Add the entire bottle contents to the vessel and mix for a minimum of 60 minutes. Adjust the pH to approximately 8.5 with 5 N or 6 N NaOH. Mix for a minimum of 10 minutes. Adjust the pH to 7.0 ± 0.5 with 5 N or 6 N HCl. Adjust to the final volume with WFI-quality water or equivalent. Mix for a minimum of 10 minutes. Filter-sterilize the solution using a 0.2 µm filter and store at 2–8°C until use. Protect from light.

Cat. No. 670108—OneFeed Supplement 50 L hydration instructions

To prepare 50 L, add 45 L of WFI-quality water or equivalent to an appropriate mixing vessel. Add the entire container contents to the vessel and mix for a minimum of 60 minutes. Adjust the pH to approximately 8.5 with 5 N or 6 N NaOH. Mix for a minimum of 10 minutes. Adjust the pH to 7.0 ± 0.5 with 5 N or 6 N HCl. Adjust to the final volume with WFI-quality water or equivalent. Mix for a minimum of 10 minutes. Filter-sterilize the solution using a 0.2 µm filter and store at 2–8°C until use. Protect from light.

Materials required but not provided

Cultures should be supplemented with cell-specific supplements as required.

Fed-batch culture process

OneFeed Supplement can be used with any existing base medium. Cells should be adapted to a complete medium prior to initiating a fed-batch culture. Cultures should be started using the seeding density typically used for the specific cell line. The recommended OneFeed Supplement protocol is to feed starting on day 2 of the culture and continue every two days during the life of the culture.

It is recommended that the amount of OneFeed Supplement fed be 5 mL of feed per 100 mL of the starting culture volume (2 g/L) as demonstrated in the example shown in the table at right. Cell viability, viable cell density, and protein production should be monitored throughout the culture life. It is recommended that glucose levels are monitored and maintained at the concentration appropriate for the cell line. OneFeed Supplement can also be used as a replacement feed using existing feeding processes.

Fed-batch cultures
(50 mL medium starting culture volume per flask)

Feed timepoint	Amount of OneFeed added per flask
Day 0	0 mL
Day 2	2.5 mL
Day 4	2.5 mL
Day 6	2.5 mL
Day 8	2.5 mL
Day 10	2.5 mL
Day 12	2.5 mL
Total amount of OneFeed added per flask	15.0 mL

Adaptation of cells

Direct adaptation

A direct adaptation procedure can be used for cells that might readily adapt into the new medium. Dilute cells growing in the current medium directly into the new medium at 5.0 x 10^5 cells/mL during the logarithmic growth phase. **Cell centrifugation and total removal of the current medium are not recommended when performing this step.** When the viable cell density reaches 1.5–2.0 x 10^6 cells/mL, or the culture is in the mid- to late-logarithmic growth phase, subculture the cells again at 5.0 x 10^5 cells/mL. Repeat the subculturing at least three times every 3 to 4 days. If the viability goes below 80% during the procedure or if the cells do not retain their normal doubling time after four subcultures, adapt the cells sequentially as follows.

Sequential adaptation

Complete adaptation from the old medium to the new medium is achieved best in a four-step process when adapting sequentially. For each step, grow the cells in the media combination for at least three days until the cells reach the mid- to late-exponential growth phase. If the culture shows longer doubling time, include additional cell passages at the media combination by following the same dilution procedure. Continue to passage the cells at least three times at the current media combination for complete adaptation and growth kinetics. We recommend preparing a cell-bank at the 50%-adapted step to provide for all contingencies.

Production can be monitored while the cells are being adapted. However, until the culture is run under the normal process conditions, the results will not be indicative of the true production performance.

Adaptation step	Seeding density	Culture conditions	Criteria for next adaptation step
25% new medium **75% old medium**	5.0 x 10^5 cells/mL	Grow at least 3 days until the cells reach mid- to late-exponential growth phase	Culture must retain normal doubling time and ≥90% viability for three passages
50% new medium **50% old medium**	5.0 x 10^5 cells/mL	Grow at least 3 days until the cells reach mid- to late-exponential growth phase	Culture must retain normal doubling time and ≥90% viability for three passages
75% new medium **25% old medium**	5.0 x 10^5 cells/mL	Grow at least 3 days until the cells reach mid- to late-exponential growth phase	Culture must retain normal doubling time and ≥90% viability for three passages
100% new medium	5.0 x 10^5 cells/mL	Allow for 3–4 passages at 100% new medium before creating cell banks and moving to the production system	The cell culture is considered adapted only when it retains normal doubling time and a minimum of 90% viability over several cell passages

Definition of methods

Analytical tests used in data gathering for this manual are described here.

The **amino nitrogen (AN)** test procedure is based on the AOAC Sorensen method.

The **AN/TN** ratio gives an estimate of the degree of protein hydrolysis.

Ash values were measured after heating at 650°C overnight. Ash values refer to the noncombustible portion of the sample and roughly correspond to the mineral content of the sample.

Total carbohydrate percentage was calculated by colorimetric assay.

Chloride, sulfate, and phosphate percentages were determined by ion chromatography.

Elemental analysis was determined by ICP (Inductively Coupled Plasma) using a Thermo Jarrell Ash instrument or equivalent.

Endotoxin values were determined by a quantitative kinetic chromogenic method.

Free amino acids are defined as amino acids that are not part of a protein or peptide chain. The amino acids were measured using the Waters AccQ•Tag™ method. The AccQ•Tag method is based on the derivatizing reagent 6-aminoquinolyl-N-hydroxysuccinimide–activated heterocyclic carbamate.

Labsystems Bioscreen C is a 200-well incubating kinetic optical density reader, and **Victor3 1420** Multilabel Counter is an optical plate reader equipped with protocols for measuring optical density on 96-well culture plates. For both systems, media were inoculated with approximately 100 CFU per 200 µL fill in each well. OD readings were averaged from four wells.

Loss on drying is a measurement of moisture in the dehydrated sample. The test procedure is based on the method described in The United States Pharmacopeia, with modifications [1].

Molecular weight distribution, which is an indication of degree of protein digestion, was determined by size-exclusion chromatography using an agarose dextran matrix–based column and a TFA/acetonitrile-based mobile phase.

Nucleoside quantitation (hypoxanthine and thymidine) was determined by reverse-phase HPLC using a silica-based column and a phosphate/methanol gradient.

pH was measured potentiometrically at room temperature in a 1% solution after autoclaving.

Sodium chloride was determined by the silver nitrate/potassium thiocyanate titration method.

Total amino acids were measured by the same method as the free amino acids after an acid hydrolysis at 110°C for 20 hours 45 minutes using a CEM microwave. Asparagine, cystine, glutamine, and tryptophan are destroyed during the hydrolysis. The asparagine, cystine, glutamine, and tryptophan values are not reported for total amino acids. Methionine and serine are partially destroyed during the hydrolysis.

Total nitrogen (TN) content is determined by the Kjeldahl method.

Ultrafiltration (UF) is a membrane filtration process used to separate or concentrate constituents of protein solutions based on molecular weight.

Reference

1. United States Phamacopeial Convention. 2006. The United States Pharmacopeia 29: (USP29): The National Formulary 23 (NF23). Rockville, MD: United States Pharmacopeial Convention Inc.

Regulatory documentation

We pride ourself on the level of regulatory support that we provide to our customers. These services have been developed based upon customer feedback and on guidance published by various international standards. We are ready to support your regulatory needs and offer the following services based on our strong commitment to quality products, reliably delivered with the appropriate documentation.

Certificates of Analysis

To streamline the communication and transmission of Certificates of Analysis (COAs) and the animal origin information they contain, we provide COAs for catalog and custom products.

COAs for catalog products are searchable by specific catalog and lot numbers on our website.

For custom products, COAs can be obtained through your regional technical support representative.

Change Notification Program

We offer an Automated Change Notification (ACN) Program to customers who require timely, detailed notification of manufacturing and process changes that comply with our quality policies. For more information on this service, please contact your regional technical support representative.

Certificates of Suitability

Where applicable, and by customer request, we hold TSE Certificates of Suitability (CEPs) issued by the European Directorate for the Quality of Medicines & Healthcare (EDQM). Inquiries regarding new or existing CEPs should be directed to your local account representative.

Product listings

Animal origin–free peptones

	100 g	454 g	500 g	1 kg	2 kg	5 lb (2.3 kg)	5 kg	10 kg	25 lb (11.3 kg)	25 kg	50 kg
Gibco Bacto Yeast Extract			212750		212720			212730			212710
Gibco Yeast Extract		211929				211930			211931		
Gibco Bacto Yeast Extract, Technical			288620					288610			
Gibco Difco Yeast Extract, UF			210929					210934			
Gibco Difco Yeast Extract Low-Dusting (LD)			210933					210941			
Gibco Bacto TC Yeastolate	255772							255771		292731	
Gibco Difco TC Yeastolate, UF			292804					292805			670079
Gibco Bacto Malt Extract			218630					218610			
Gibco Phytone Peptone		211906				298147		292450			
Gibco Difco Phytone Supplement UF			210931					210936			
Gibco Difco Soytone			212488					212489			
Gibco Bacto Soytone*			243620					243610			
Gibco Soy Peptone 100			670138					670137			
Gibco Wheat Peptone 100			670140					670139			
Gibco Cotton Peptone 200 UF			670104				670105				

Animal origin peptones

	100 g	454 g	500 g	1 kg	2 kg	5 lb (2.3 kg)	5 kg	10 kg	25 lb (11.3 kg)	25 kg	50 kg
Gibco Beef Extract Powder			212303								
Gibco Bacto Beef Extract, Desiccated			211520								
Gibco Difco Beef Extract			212610								
Gibco Gelysate Peptone		211870									
Gibco Bacto Neopeptone			211681					211680			
Gibco Bacto Peptone			211677		211820			211830			
Gibco Polypeptone Peptone		211910						297108			
Gibco Bacto Proteose Peptone			211684					212010			
Gibco BiTek Proteose Peptone								253310			
Gibco Bacto Proteose Peptone No. 2			212120					212110			
Gibco Bacto Proteose Peptone No. 3			211693		212220			212230			211692
Gibco BiTek Proteose Peptone No. 3										253720	
Gibco Bacto Proteose Peptone No. 4								211715			
Gibco Bacto Tryptose			211713					211709			
Gibco Acidicase Peptone			211843								
Gibco Bacto Casamino Acids			223050		223020			223030			
Gibco Bacto Casamino Acids, Technical			223120					223110			
Gibco Difco Casamino Acids, Vitamin Assay	228820		228830								
Gibco Biosate Peptone		211862							294312		
Gibco Difco Casein Digest			211610								
Gibco Bacto Casitone			225930					225910			
Gibco Difco Tryptone		211921				211922			211923		
Gibco Bacto Tryptone			211705		211699			211701			
Gibco BiTek Tryptone								251420			
Gibco Bacto TC Lactalbumin Hydrolysate			259962					259961			

Chemically defined supplements and feeds

	100 g	1 kg	5 kg
Gibco Recharge Supplement without Glucose and L-Glutamine	670002	670003	670004
Gibco Resurge CD1	670011	670012	670013
Gibco Resurge CD2	670015	670016	670017
Gibco Resurge CD3	670018	670019	670020
Gibco Resurge CD4	670021	670022	670023
Gibco Resurge CD5	670024	670025	670026
Gibco Resurge CD Pak (100 g x 5)	670030		

	Powder		
	2 L	10 L	50 L
Gibco OneFeed Supplement	670110	670109	670108

Microbial and vaccine media

	10 kg
Gibco Bacto Tryptose Phosphate Broth	260200
Gibco Difco PPLO Broth	255410
Gibco Difco Malt Extract Broth	214912
Gibco Bacto Brain Heart Infusion	237300
Gibco Bacto Brain Heart Infusion, Porcine	256110
Gibco Difco Brain Heart Infusion, without Dextrose	250220

Notes

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