

Thermo Scientific Nunc Immobilizer Nickel-Chelate

Instruction protocol

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The Thermo Scientific™ Nunc™ Immobilizer™ Nickel-Chelate plates are manufactured using a patented photochemical method¹ for covalent coupling of ligands to polymer surfaces.

A Nickel-Chelate complex is coupled via a spacer to the plates. The Nunc Immobilizer Nickel-Chelate Plates are designed for optimal binding of fusion proteins that have been tagged with an amino sequence containing six repetitive histidine (His) molecules.

Materials:

- Nunc Immobilizer Nickel-Chelate Plates
- His-tagged biomolecule

Reagents:

- Coupling buffer: 0.01 M KCl
- Washing buffer: PBST (Phosphate Buffered Saline pH 7.3 containing 0.05% (v/v) TWEEN® 20)

Coupling protocol for 96 well plate:

1. Pre-wash the plate with PBST (3 x 300 µl/well)
2. Prepare a solution of a His-tagged protein (0.01-1 µg/ml) in 0.01M KCl
3. Add the protein solution to the wells of the Nunc Immobilizer Nickel-Chelate Plate (100 µl/well)
4. Incubate the plate with gentle agitation (100 rpm) at 20-25°C for 2 hours, or at +4°C overnight
5. Aspirate the wells and wash with PBST (3 x 300 µl/well)
6. The plate is now ready for your assay

Coupling protocol for 384 well microplate:

1. Pre-wash the plate with PBST (3 x 100 µl/well)
2. Prepare a solution of a 6 x His-tagged protein (0.01-1 µg/ml) in 0.01M KCl
3. Add the protein solution to the wells of the Nunc Immobilizer Nickel-Chelate Plate (50 µl/well)
4. Incubate the plate with gentle agitation (100 rpm) at 20-25°C for 2 hours, or at +4°C overnight
5. Aspirate the wells and wash with PBST (3 x 100 µl/well)
6. The plate is now ready for your assay

Additional comments and recommendations

It is recommended to include small amounts of a non-ionic detergent like TWEEN 20 (0.05% (v/v)) in washing buffers and in assay buffers, as this generally improves the signal to noise ratio of the assay.

Stability

Nunc Immobilizer Nickel-Chelate Plates can be stored at room temperature to their expiration date, which appears on the case label.

Trademarks and Patents:

TWEEN 20 is a registered trademark of ICI American Inc., U.S.A. Immobilizer is a trademark of Exiqon A/S, Vedbaek, Denmark.

The product is produced under license from Exiqon A/S - EP 08 20483 and foreign applications and patents.

References:

- ¹ Jensen, S. P., Rasmussen, S. E., Jakobsen, M. H. Photochemical Coupling of Peptides to Polystyrene MicroWell Plates. *Innovations & Perspectives in Solid Phase Synthesis & Combinatorial Chemical Libraries*, (1996), 419-422.

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