Thermo Scientific Nunc Immobilizer Amino
Instruction protocol

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The Thermo Scientific™ Nunc™ Immobilizer™ Amino Plates/Strips are manufactured using a patent photochemical method[1] for covalent coupling of ligands to polymer materials. The photocoupling introduces an ethylene glycol spacer and a stable electrophilic group that reacts with nucleophiles such as free amines, thiols or hydroxy-groups. The spacer design and the density of electrophilic groups on this surface are optimized for peptide and protein based immuno-diagnostic assays.

Materials

- Nunc Immobilizer Amino Plates/Strips
- Peptide, protein or antibody of choice

Reagents

- Peptide coupling buffer: 100 mM sodium carbonate buffer, pH 9.6
- Protein coupling buffer: 100 mM sodium phosphate 5.8-8.02 pH (depending on the properties of the protein) or 1 X PBS (Phosphate Buffered Saline,10 mM Sodium Phosphate Buffer, pH 7.5, 150 mM NaCl) or 100 mM sodium carbonate, pH 9.6
- Antibody coupling buffer: 100 mM sodium phosphate, pH 8.0
- Washing buffer: PBST, pH 7.2 (Phosphate Buffered Saline containing 0.05% (v/v) TWEEN® 20)

Note: Buffers should be used within one week from preparation.

Recommended protein and antibody coupling protocol

1. Prepare a solution of your peptide (1-100 µg/ml) in 100 mM carbonate buffer pH 9.6
2. Add the peptide solution to the wells of the Nunc Immobilizer Amino:
   For 96 well plate and 8 well strips: 100 µl/well
   For 384 well plate: 50 µl/well
3. Incubate the plate with gentle agitation at room temperature (20°-25°C) for 1-2 hours or overnight at +4-8°C
4. Aspirate the wells and wash with PBST:
   For 96 well plate and 8 well strips: 3 x 300 µl
   For 384 well plate: 3 x 100 µl
5. Your peptide surface is ready for use

Recommended protein and antibody coupling protocol

1. Prepare a solution of your protein or antibody (0.01-100 µg/ml) in the preferred coupling buffer
2. Add the solution to the wells of the Nunc Immobilizer Amino Plate/Strips:
   For 96 well plate and 8 well strips: 100 µl/well
   For 384 well plate: 50 µl/well
3. Incubate the plate/strips with gentle agitation at room temperature (20-25°C) for 1-2 hours or overnight at +4-8°C
   Aspirate the wells and wash with PBST
   For 96 well plate and 8 well strips: 3 x 300 µl
   For 384 well plate: 3 x 100 µl
4. Your protein or antibody surface is ready for use

Additional Comments and Recommendations

To find the optimal coupling concentration, it is suggested that a dilution series of the molecule of interest be initially run in the recommended coupling buffers. A suitable concentration at which to start is around 100 µg/ml. Coupling times vary and should be determined empirically, but a good starting place is a 1 hour incubation at room temperature (20-25°C). Amines, other nucleophiles, or non ionic detergents like TWEEN 20 should not be present in the coupling buffer.
For the washing and assay buffers the use of a low concentration of a non-ionic detergent like TWEEN 20 (0.05%-2%) (v/v)) is recommended, as it generally improves the signal to noise ratio for the assay. Additional improvement in the assay signal to noise ratio may be gained by treating the plate post coupling with ethanolamine. This can be done by adding 10 mM ethanolamine in 100 mM Na-Carbonate, pH 9.6, buffer to the wells and incubating for 1 hour at room temperature (20 to 25°C). This makes the surface more hydrophilic and thus less prone to non-specific adsorption.
Using plates prepared in the manner described above, it is not necessary to add a carrier protein such as BSA or gelatin to your assay buffer.

Stability

Nunc Immobilizer Amino 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.

[2] Sambrook, J., Fritsch, E.F., Maniatis, T.: Molecular Cloning, A Laboratory Manual 2nd edition. Cold Habor Laboratory Pres., Cold Spring Habor, NY (1989), Appendix B.21.

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