# Thermo Scientific Nunc Immobilizer Glutathione

## Instruction protocol

#### Thermo Scientific Nunc Immobilizer Glutathione

The Thermo Scientific™ Nunc™ Immobilizer™ Glutathione Plates are manufactured using a patented photochemical method¹ for covalent coupling of ligands to polymer surfaces.

Glutathione is coupled via a spacer to the plate surface.

The Nunc Immobilizer Glutathione Plates are designed for optimal binding of glutathione-S-transferase (GST)-tagged proteins.

#### **Materials:**

- Nunc Immobilizer Glutathione Plates
- · GST-tagged protein of choice

#### Reagents:

- Coupling buffer: PBS (Phosphate Buffered Saline), pH=7.2
- Washing buffer: PBST (Phosphate Buffered Saline containing 0.05% (v/v) TWEEN® 20)

## Coupling protocol for 96 well plate:

- Prepare a solution of your purified GST or a GST- tagged protein (0.01-1 μg/ml) in PBS Note! Do not whirl mix, as the GST-tagged molecule will degrade and prohibit binding of the glutathione
- 2. Add the protein solution to the wells of the Nunc Immobilizer Glutathione plate (100 ul/well)
- 3. Incubate the plate/strips with gentle agitation (e.g. 100 rpm) at room temperature (20° 25°C) for 2 hours, or overnight at +4°C
- 4. Aspirate the wells and wash with PBST (3 x 300 µl)
- 5. The plate is now ready for your assay

### Coupling protocol for 384 well plate:

- Prepare a solution of your purified GST or a GST-tagged protein (0.01-1 μg/ml) in PBS. Note! Do not whirl mix, as the GST-tagged molecule will degrade and prohibit binding of the glutathione
- 2. Add the above solution to the wells of the Nunc Immobilizer Glutathione Plate (50  $\mu$ I/well)
- Incubate the plate with gentle agitation (e.g. 100 rpm) at room temperature (20° 25°C) for 2 hours, or overnight at +4°C
- 4. Aspirate the wells and wash with PBST (3 x 100 µl/ well)
- 5. The plates are now ready for your assay

#### **Additional comments and recommendations**

It is not necessary to block the plates. During the coupling of GST (Steps 1, 2, and 3 of the protocols) non-ionic detergents like TWEEN 20 should NOT be present, as these will decrease the coupling of purified GST or GST-tagged fusion proteins.

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 Glutathione 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:

<sup>1</sup> 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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