Application Note

Thermo Scientific Nunc Immobilizer Glutathione

Key Words

Thermo Scientific™ Nunc™ Immobilizer™ Glutathione MicroWell™ plate, glutathione-S-transferase, GST-tagged proteins, recombinant fusion proteins.

Goal

The goal of this application note is to show the optimal protocol for the Immobilizer Glutathione plate. Further to show that this will results in a high signal to noise ratio and a low detection limit due to the special concept of the plates.

Nunc Immobilizer Glutathione MicroWell plate is manufactured using a patented photochemical method[1] for covalent coupling of ligands to polymer surfaces via a spacer. The Nunc Immobilizer Glutathione MicroWell plates are designed for optimal binding of glutathione-S-transferase (GST)-tagged proteins.

Recommended coupling protocol

Materials

- Nunc Immobilizer Glutathione MicroWell plates
- Coupling buffer: PBS (Phosphate Buffered Saline), pH 7.2
- Washing buffer: PBST (Phosphate Buffered Saline containing 0.05% (v/v) Tween 20).

Protocol for 96 well plates

1. Prepare a solution of purified GST or a GST-tagged protein (0.001-1 µg/mL) in PBS.
   NB! Do not whirl mix.
2. Add the protein solution to the wells of the Nunc Immobilizer Glutathione plate (100 µL/well).
3. Incubate with gentle agitation (100 rpm) at room temperature for two hours or overnight at +4°C.
4. Aspirate the wells and wash with PBST (3 x 300 µL).

Protocol for 384 well plates

1. Prepare a solution of purified GST or a GST-tagged protein (0.001-1 µg/mL) in PBS.
   NB! Do not whirl mix.
2. Add the protein solution to the wells of the Immobilizer Glutathione plate (50 µL/well).
3. Incubate the plate with gentle agitation (100 rpm) at room temperature for two hours or overnight at +4°C.
4. Aspirate the wells and wash with PBST (3 x 100 µL).

It is not necessary to block the plates. During the coupling of GST (step 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.

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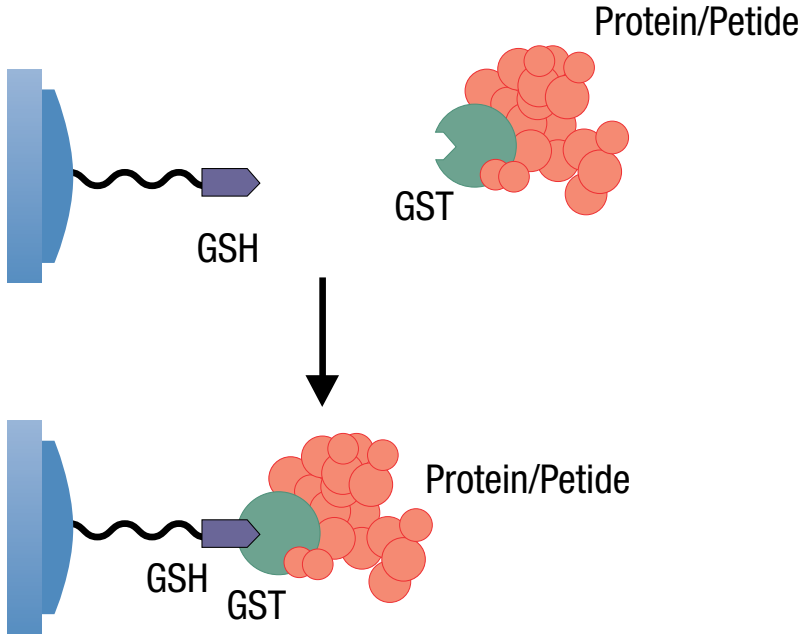

Fig. 1.

Coupling of a GST-tagged protein/peptide to the Immobilizer Glutathione plate

It is recommended to include small amounts of a non-ionic detergent like Tween 20 (0.05% (v/v)) in washing buffers and in buffers for dilution of antibodies (e.g. anti-GST, antibodies against the fusion protein, secondary antibodies), as this generally improves the signal to noise ratio of the assay.

Application example

Assay for determination of a GST-tagged fusion protein

Performance of Immobilizer Glutathione plates is illustrated using the transparent 96 well plate.

The Immobilizer Glutathione plate is ready to use. A GST-tagged fusion protein (56 kDa) is applied to the plate in a series of dilutions. The amount of immobilised GST-tagged fusion protein is detected by addition of an antibody against GST conjugated to horseradish peroxidase (HRP).

The amount of HRP is measured by addition of a substrate/chromogen (e.g. TMB). The color development based on the enzyme activity of the HRP is proportional to the amount of immobilised GST.

The reaction is stopped by adding stop solution (e.g. 1 N H^2SO^4).

The following assay is performed on the Immobilizer Glutathione plate.

GST-tagged fusion protein determination

1. Prepare a series of GST-tagged fusion protein solutions in PBS (0.005-0.05 µg/mL).
2. Dispense 100 µL of each of these solutions into the wells of a transparent Immobilizer Glutathione plate rows 1-10. PBS is added to rows 11-12 as negative controls.
3. Incubate the plate with gentle agitation (100 rpm) at room temperature for two hours.
4. Aspirate the wells and wash with PBST (3 x 300 µL).
5. Add an anti-GST antibody or an antibody against the fusion protein diluted in PBST to the plate (100 µL/well) to rows 1-11. PBST is added to row 12 as a negative control (result not shown).
6. Incubate the plate with gentle agitation (100 rpm) at room temperature for one hour.
7. Aspirate the wells and wash with PBST (3 x 300 µL).
8. If the antibodies are not conjugated with HRP, an appropriate HRP conjugated antibody must be added.
9. Incubate the plate with gentle agitation (100 rpm) at room temperature for one hour.
10. Aspirate the wells and wash with PBST (3 x 300 µL).
11. Add TMB solution to the plate (100 µL/well).
12. Incubate the plate for 10 minutes in the dark.
13. Add 1 N H^2SO^4 to the plate (100 µL/well).
14. Read the absorbance at 450 nm.

As can be seen in Fig. 2, the background is extremely low using the Immobilizer Glutathione plate. This results in a high signal to noise ratio and a low detection limit, which in this example is 3 ng GST-tagged fusion protein per mL (0.3 ng/well) assuming OD cut off value of 0.5.

Furthermore, the assay time is very short on the Immobilizer Glutathione plate for detection of GST-tagged fusion proteins. This is due to fewer incubation steps in the assay (e.g. omitting the blocking steps).

Credits

Assay and protocol for Glutathione plate preparation was designed by Eva Jauho.

References

1. Jensen SP, Rasmussen SE, Jakobsen MH.
Photochemical Coupling of Peptides to Polystyrene MicroWell Plates.
Innovations & Perspectives in Solid Phase Synthesis & Combinatorial Chemical Libraries (1996), 419-422.

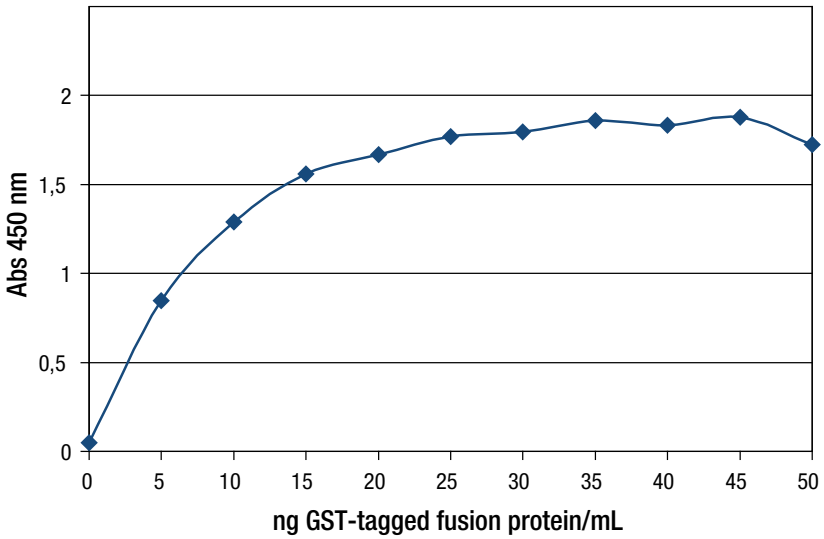

Fig. 2.

Dilution of a GST-tagged fusion protein (56 kDa) in PBS.

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