Detection of glutathione-S-transferase (GST) and GST-tagged fusion (F-protein)

Key Words

Thermo Scientifc™ Nunc™ Immobilizer™ plates, coated plates, GST binding, Purified GST, ELISA, recombinant fusion proteins, no blocking steps.

Goal

The goal of this application note is to show the Immobilizer Glutathione plate can both be used for purified GST but also for non-purified GST-tagged fusion proteins. 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.

The Nunc Immobilizer Glutathione plates are designed for optimal binding of GST and GST-tagged F-proteins.

No purification of GST-tagged fusion proteins is needed. Detection limit is very low.

The functional glutathione surface is based on patented technology for covalent coupling of ligands to polymer surfaces [1] via a spacer.

Principle of the GST detecting assay

No blocking steps are required prior to addition of GST or detecting antibody.

The GST/GST-tagged fusion protein (F-protein) detection assay is conducted as follows:

- Purified GST or GST-tagged F-protein is applied to the plates. It is recommended to include controls without GST.
- The plates are incubated and subsequently washed to remove unbound proteins.
- The bound concentrations of the GST are detected as follows:
    1. addition of an enzyme conjugated antibody against GST or the protein part of the GST-tagged F-protein, or
    2. addition of an antibody against GST or the F-protein followed by addition of an enzyme conjugated antibody against the anti-GST or the F-protein antibody.
- The plates are incubated and subsequently washed to remove unbound proteins after each antibody application.
- The amount of bound enzyme (i.e. bound enzyme conjugated antibody) is visualized by addition of a chromogen/substrate solution.
  The enzyme reaction is stopped by addition of stop solution.
- The enzyme reactivity of the plates is measured in an ELISA reader.

Recommended protocol for the detection of His-tagged F-proteins

Reagents and materials

- Nunc Immobilizer Glutathione plate
- Coupling buffer: PBS (Phosphate Buffered Saline) (pH 7.2)

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- Washing buffer: PBST (Phosphate Buffer Saline (pH 7.2) containing 0.05% (v/v) Tween 20
- Chromogen/substrate: 3,3’, 5,5’ tetramethylbenzidine (TMB)
- Stop solution: Sulphuric acid 0.5 M

Application examples

Detection of GST-tagged F-proteins

The following assay was conducted:

- A GST-tagged F-protein (35 kDa) expressed in an unpurified cell lysate (100 µL/well) was applied to the Immobilizer Glutathione plates in ten concentrations in a 1:2 dilution series (dilutions from 2000 ng/mL to 3.9 ng/mL).
  A negative control without GST was also added to the plates.
- The plates were incubated for two hours at room temperature with gentle agitation (100 rpm).
- The plates were washed three times with 300 µL/well PBST.
- Anti-GST (100 µL/well) diluted 1:2000 in PBST was added to the plates.
- The plates were incubated for one hour at room temperature with gentle agitation (100 rpm).
- The plates were washed three times with 300 µL/well PBST.
- TMB (100 µL/well) was added to the plates.
- The plates were incubated for 10 minutes at room temperature in the dark.
- The enzyme reaction was stopped with 0.5 M sulfuric acid (100 µL/well).
- The absorbance was read at 450 nm.

Fig. 1 illustrates the OD response from an 1:2 dilution curve of an unpurified GST-tagged F-protein applied to a Glutathione Immobilizer plate.

As seen in Fig. 1, the detection limit for an unpurified GST-tagged F-protein is very low. Assuming a cut-off OD value of 0.5, the detection limit is 1.5 ng/well.

Even when testing an unpurified preparation of a cell lysate using the Immobilizer Glutathione plates very small concentrations of GST-tagged F-proteins can be detected. In the control without GST-tagged F-protein the background is very low (OD value 0.046).

The GST-tagged F-protein was kindly provided by Dr. Karsten Kristiansen, Syddansk University, Odense, Denmark.

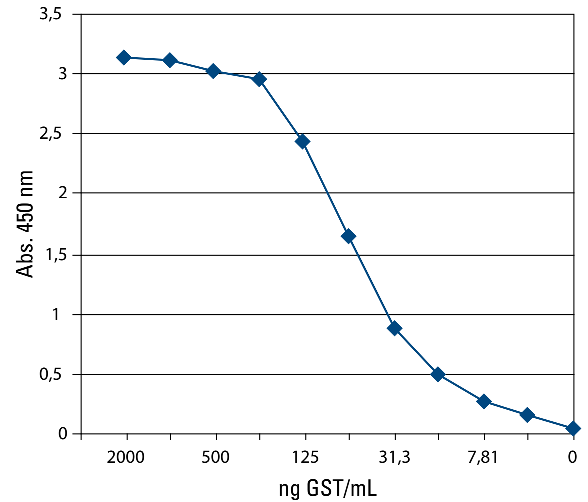

Fig. 1.

Detection of a GST-tagged fusion protein from an unpurified cell lysate. The GST-tagged fusion protein was applied in 1:2 dilutions from 2000 ng/mL to 3.91 ng/mL. As a negative control, buffer without GST-tagged fusion protein was applied.

Detection of GST

The following assay was conducted:

- Purified GST (100 µL/well) was added to the Immobilizer Glutathione plates at ten concentrations in a 1:2 dilution series (2000 ng/mL to 3.9 ng/mL).
  A negative control without GST was also added to the plates.
- The plates were incubated for two hours at room temperature with gentle agitation (100 rpm).
- The plates were washed three times with 300 µL/well PBST.
- Anti-GST (100 µL/well) diluted 1:2000 in PBST was applied to the plates.
- The plates were incubated for one hour at room temperature with gentle agitation (100 rpm).
- The plates were washed three times with 300 µL/well PBST.
- A chromogen/substrate solution TMB (100 µL/well) was applied to the plates.
- The plates were incubated for 10 minutes at room temperature in the dark.
- The enzyme reaction was stopped with 0.5 M sulfuric acid (100 µL/well).
- The absorbance was read at 450 nm.

Fig. 2 illustrates the OD response from a 1:2 dilution curve of purified GST applied to a Immobilizer Glutathione plate. Assuming a cut-off OD value of 0.5, the detection limit is 2.5 ng/well.

The detection limit for purified GST is thus very low. As seen from the control without GST the background is also very low (OD value 0.045).

Reproducibility of the Glutathione Immobilizer MicroWell plates

Four Glutathione Immobilizer plates (two different plate batches A and B) were tested. Purified GST was applied to the plates in different dilutions.

Assay procedure 96 well transparent plates

- Purified GST or GST-tagged F-proteins (100 µL/well) is applied to the Glutathione Immobilizer plates in concentrations from 300 ng/mL to 30 ng/mL. A control without GST is also applied to the plates.
- The plates are incubated for two hours at room temperature with mild agitation (100 rpm).
- The plates are washed three times with 300 µL/well PBST.
- Anti-GST (100 µL/well) diluted 1:2000 in PBST is applied to the plates.
- The plates are incubated for one hour at room temperature with mild agitation (100 rpm).
- The plates are washed three times with 300 µL/well PBST.
- TMB (100 µL/well) is applied to the plates.
- The plates are incubated for 10 minutes at room temperature in the dark.
- The enzyme reaction is stopped with 0.5 M sulfuric acid (100 µL/well).
- The absorbance was read at 450 nm using an ELISA reader.

Fig. 3 illustrates the OD response from four dilution curves of purified GST applied to four Immobilizer Glutathione plates.

As seen in Fig. 3, the performance of the plates shows very little variation.

Features and benefits of the Immobilizer Glutathione plates

- No steric hindrance for GST-tagged fusion proteins.
- No blocking or activation steps needed.
- No purification of GST-tagged fusion proteins needed.
- Short assay time (3½ hours).
- Low detection limits.
- To date we can document nine month stability at 20-25°C.

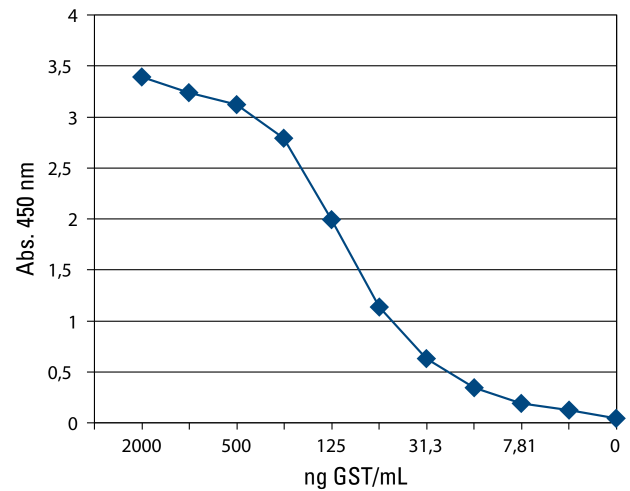

Fig. 2.

Detection of purified GST. Purified GST was added in 1:2 dilutions from 2000 ng/mL to 3.91 ng/mL. As a negative control, buffer without GST was applied.

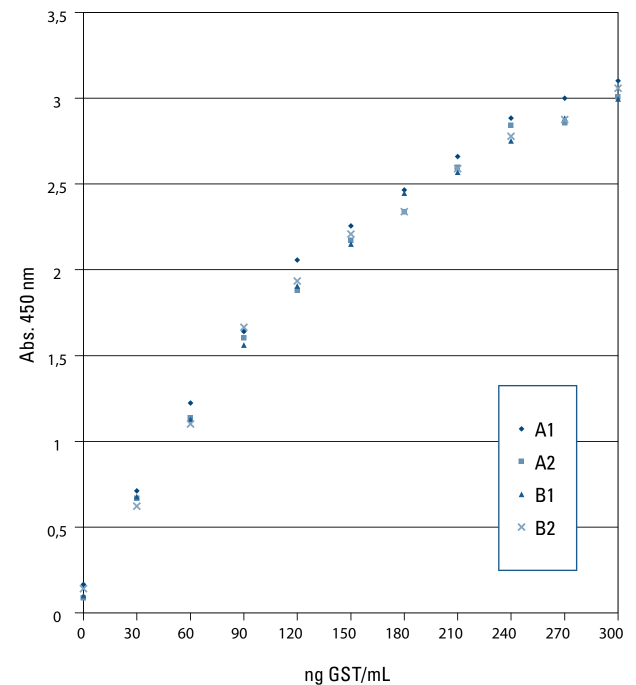

Fig. 3.

Four Immobilizer Glutathione plates from two different plate batches (A and B) were tested. Purified GST was applied to the plates in the indicated concentrations.

Credits

Assay and protocol for Glutathione plate preparation was designed by Eva Jauho, Betina Jacobsen and Maria Nielsen.

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