Application Note

Thermo Scientific Nunc Immobilizer Glutathione and Nickel-Chelate

Key Words

Thermo Scientific™ Nunc™ Immobilizer™ Glutathione, Thermo Scientific™ Nunc™ Immobilizer™ Nickel-Chelate, GSH, Ni-Chelate, GST-tagged proteins, His-tagged proteins, recombinant fusion proteins, low detection limit, high signal to noise, and high reproducible results.

Goal

The goal of this application note is to show the Immobilizer Glutathione plate and Immobilizer Ni-Chelate via a simple one-step protocol can bind either glutathione-S-transferase (GST)-tagged fusion proteins, purified GST or crude/purified His-tagged fusion proteins. Further to confirm the features of high signal to noise ratio, very low detection limits and high reproducible results for these plates.

Binding of tagged fusion proteins

Glutathione (GSH) is a tri-amino peptide, which is covalently attached to the polystyrene using unique surface chemistry. Via a simple one-step protocol it can bind with glutathione-S-transferase (GST)-tagged fusion proteins or purified GST. Nunc Immobilizer Glutathione can, for example, be used for the analysis of a cell lysate to determine the presence and concentration of a desired GST-tagged protein. The Nunc Immobilizer Nickel-Chelate is a similar system that is designed for the determination of 6 x His-tagged fusion proteins. Both of these products can be used with crude extracts, as well as purified cell material.

In common with the other Immobilizer products, the placement of the reactive groups on a spacer allows interaction, even with large proteins, with very little to no steric hindrance.

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Common features

No blocking steps

The monolayer coating on the plates provides a hydrophilic surface that requires no subsequent blocking steps. Immobilizer surfaces are optimized to ensure minimal steric hindrance of binding. This is due to the hydrophilic linker that provides an optimal distance from the surface.

High signal to noise ratio

High signal readouts and low background (see Figs. 1 and 3) characterize the Immobilizer products even when testing low sample concentrations. These features enchance the sensitivity and specificity of the plates and in combination with no or very low non-specific binding, ensure superior signal to noise ratios.

As can be seen from Fig. 2, the detection limit is extremely low when using the Immobilizer Nickel-Chelate plate. This results in a detection limit, which in this assay is 4 ng 6 x His-tagged fusion protein per mL (0.4 ng/per well) when assuming OD = 0.5 as a significant response (cut off value) relative to the background.

Mechanism of action

Immobilizer Glutathione

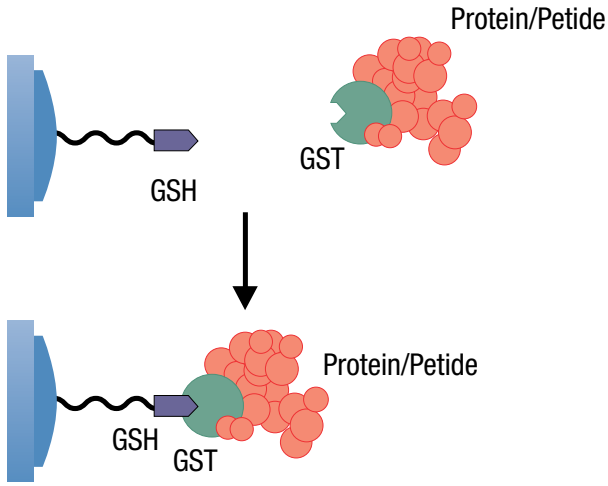


Fig. 1

The signal obtained on the Immoblizer Glutathione plates was compared to glutathione plates from competitors A and B. A purified GST protein was applied on the plates in different dilutions, following the supplier's protcols. The amount of bound GST was detected by addition of an anti-GST antibody conjugated to HRP (Horseradish Peroxidase) followed by TMB and read at 450 nm. The Immobilizer plate shows high signal readout, even at very low sample concentrations and minimal background compared to the plates from competitors.

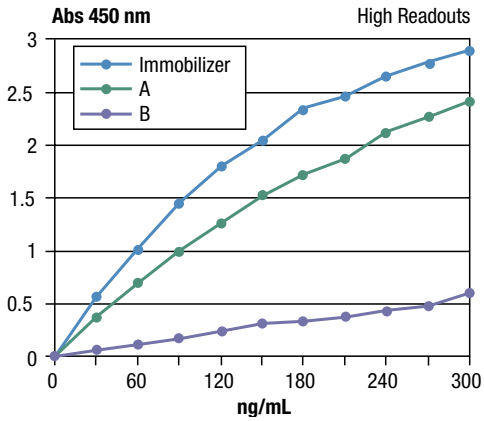
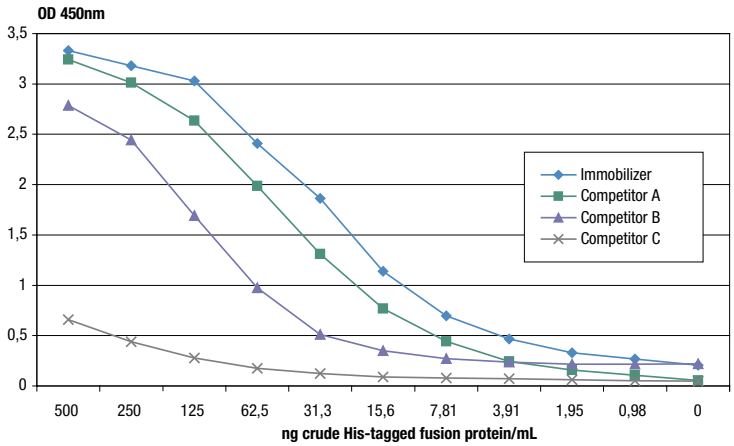


Fig. 2

Detection of a His-tagged fusion protein from a crude cell lysate preparation (crude His-ß-2-m)



Very low detection limits

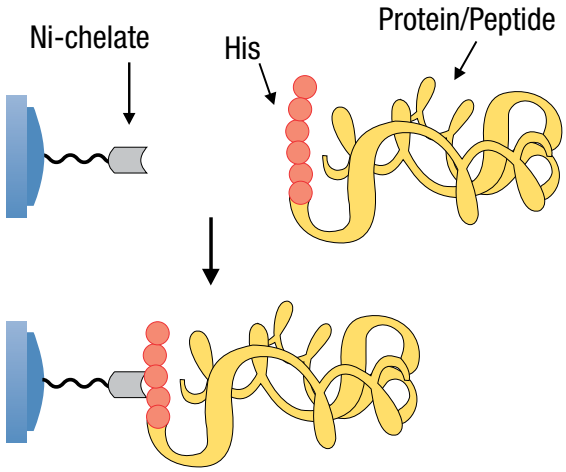
The recommended coupling protocols are extremely simple and the required concentrations of target molecule in the coupling buffer are usually low. The detection limit for GST-tagged protein, assuming a cutoff OD value of 0.5, is 1.5 ng/well (see Fig. 3). For Nickel-Chelate the detection limit, under the same assumption, is 0.4 ng/well.

Highly reproducible results

Assays conducted on the Immobilizer plates are characterized by high reproducibily (see Fig. 4). The covalently bound monolayer coating of the plates is highly uniform. This feature makes the Immobilizer plates the preferred choice in critical assay procedures.

Mechanism of action

Immobilizer Nickel-Chelate



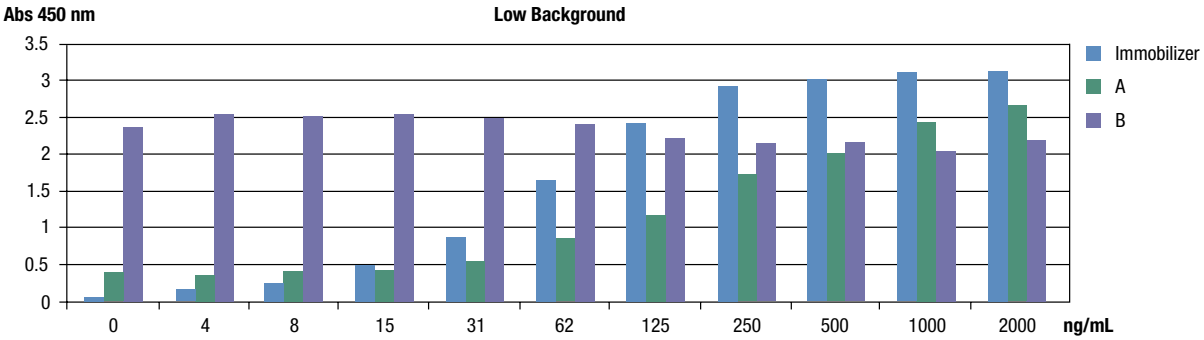
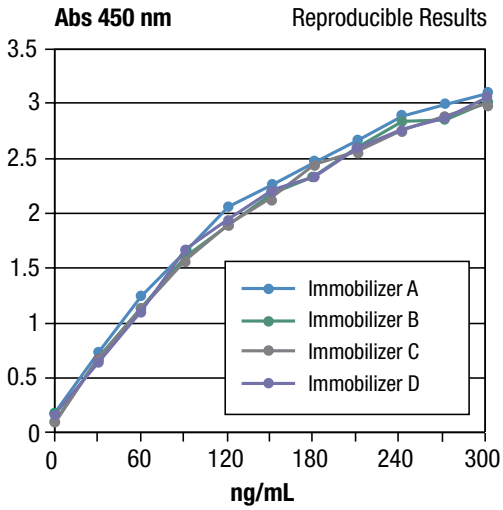


Fig. 3

The figure shows the OD response of GST-tagged fusion protein applied to Immobilizer Glutathione, competitors A and B in different concentrations (following the protocols supplied). The amount of bound GST-tagged protein was detected with an anti-GST antibody conjugated to HRP (Horseradish Peroxidase) followed by TMB and read at 450 nm. The results show that the background is much lower on the Immobilizer plate than on the competitors plates. The Immobilizer Glutathione shows a very low detection limit for GST-tagged protein. The detection limit (OD = 1) for Glutathione Immobolizer is 40 ng/mL (4 ng/well, data on file) in this assay.

Fig. 4

Dilutions of purified GST tested on four different Immobilizer Glutathione plates (A, B, C and D) from two different batches. The amount of bound GST was detected by addition of an anti-GST antibody conjugated to HRP (Horseradish Peroxidase) followed by TMB and read at 450 nm.



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ANZ: Australia: 1300 735 292, New Zealand: 0800 933 966; **Asia:** China Toll-free: 800-810-5118 or 400-650-5118;
India: +91 22 6716 2200, India Toll-free: 1 800 22 8374; Japan: +81-3-5826-1616; Other Asian countries: 65 68729717
Europe: Austria: +43 1 801 40 0; Belgium: +32 2 482 30 30; Denmark: +45 4631 2000; France: +33 2 2803 2180;
Germany: +49 6184 90 6000, Germany Toll-free: 0800 1-536 376; Italy: +39 02 95059 554;
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North America: USA/Canada +1 585 586 8800; USA Toll-free: 800 625 4327
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