Application Note

Detection of His-tagged fusion proteins

Key Words

Thermo Scientific™ Nunc™ Immobilizer™ Ni-Chelate plates, coated plates, ELISA, His-tagged protein, fusion proteins, competitor analysis, low detection limit, high signal/noise ratio.

Goal

The goal of this application note is to is to detect His-tagged fusion proteins in Nunc Immobilizer Ni-Chelate plates compared to competitor plates.

The Nunc Immobilizer Ni-Chelate plates are designed for optimal binding of His-tagged fusion proteins without the need for purification prior to application.

Detection limit is very low. The functional Ni-Chelate surface is based on patented technology for covalent coupling of ligands to polymer surfaces[1] via a spacer.

Detection of histidine (His)-tagged fusion proteins on the Nunc Immobilizer Ni-Chelate plate compared with competitor plates.

Recommended protocol for the detection of His-tagged fusion proteins

Reagents and materials

- Nunc Immobilizer Ni-Chelate plate
- Coupling buffer: 0.01 M KCl
- Washing buffer: PBST (Phosphate Buffered 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

Assay procedure 96 well transparent plates

- The plates are pre-washed three times with 300 µL/well PBST.
- Purified or crude His-tagged fusion protein (100 µL/well) is added to the Immobilizer Ni-Chelate plates in concentrations from 0.01 to 1 µg/mL. A control without His-tagged fusion protein is also included.
- The plates are incubated for two hours at room temperature with gentle agitation (100 rpm).
- The plates are washed three times with 300 µL/well PBST.
- Antibody against the fusion protein conjugated to horseradish peroxidase (HRP) (100 µL/well) diluted e.g. 1:5000 in PBST is added to the plates.
- The plates are incubated for one hour at room temperature with gentle agitation (100 rpm).
- The plates are washed three times with 300 µL/well PBST.
- TMB (100 µL/well) is added to the plates.
- The plates are incubated for 10 minutes at room temperature in the dark.

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- The enzyme reaction is stopped with 0.5 M sulfuric acid (100 µL/well).
- The absorbance is read at 450 nm using an ELISA reader.

Application example

A comparison of the performance of the Immobilizer Ni-Chelate plate and other commercially available plates was performed.

Three different preparations (A, B, C) of a fusion protein b-2-microglobulin (b-2-m 12 kDa) tagged with six His residues were tested.

A. A crude preparation of b-2-m tagged with a sequential His tag (crude His-b-2-m).

B. A purified preparation of b-2-m tagged with a sequential His tag (purified His-b-2-m).

C. A purified preparation of b-2-m tagged with an non-sequential His tag (purified HAT-b-2-m). The protein was tagged with a sequence of 20 amino acid, which included 6 histidines uniformly distributed throughout the sequence.

The following assay was conducted on the Immobilizer Ni-Chelate plate:

- The plates were pre-washed three times with 300 µL/well PBST.
- A His-tagged fusion protein (A, B or C) was applied to the Immobilizer Ni-Chelate plates (100 µL/well) in ten concentrations of a 1:2 dilution (500 ng–0.98 ng/mL).
- 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.
- Antibody against the fusion protein conjugated with HRP (HRP conjugated to anti-b-2-m) diluted in PBST was added to the plates (100 µL/well). The antibody was diluted to an appropriate concentration, here 1:5000.
- 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 +20-25°C in the dark.
- The enzyme reaction was stopped with 0.5 M sulfuric acid (100 µL/well).
- The absorbance was read at 450 nm.

The assays on the competitor plates were performed using the protocol supplied by the different competitors.

Figs. 1 and 2 illustrate the OD response of a 1:2 dilution curve of the same fusion protein (crude His-b-2-m or purified His-b-2-m) applied to a Immobilizer Ni-Chelate plate, as well as to plates from competitors A, B and C.

As seen in Figs. 1 and 2 the performance of the Immobilizer Ni-Chelate plate is superior to the performance of the competitor plates regardless of the degree of purification of the His-tagged fusion protein.

Even when testing an unpurified preparation of a cell lysate using the Immobilizer Ni-Chelate plates, very small concentrations of His-tagged fusion protein (0.5 ng per well) can be detected assuming an OD cut off value of 0.5.

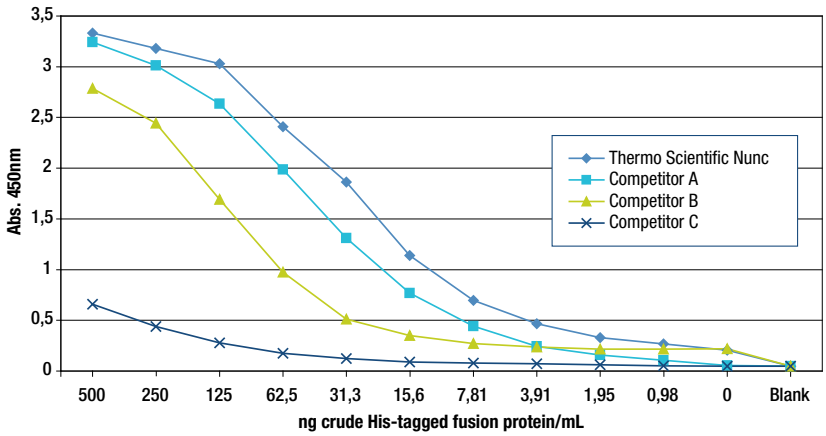

Fig. 1.

Detection of a His-tagged fusion protein from a crude cell lysate preparation (crude His-ß-2-m, preparation A). The His-tagged fusion protein was added in a 1:2 dilution to the plates. As a negative control, buffer without His-tagged fusion protein was added.

Fig. 3 illustrates a 1:2 dilution curve of the fusion protein tagged with an non-sequential His tag (purified HAT-b-2-m, preparation C). The purified HAT-b-2-m was applied to a Immobilizer Ni-Chelate plate, as well as to plates from competitors A, B and C. As seen in Fig. 3, the performance of the Immobilizer Ni-Chelate plate is superior to the performance of the competitor plates.

Conclusion

As seen from Figs. 1 to 3, the Immobilizer Ni-Chelate plate shows a higher OD response when compared to the competitor plates. Also the calculated CV% is lower on the Immobilizer Ni-Chelate plate than on many competitor plates (data not shown).

This finding is true regardless of the histidine tagged fusion protein used (His in sequence or in an non-sequential distribution as well as a crude preparation of the His-tagged fusion protein).

Even when a fusion protein is tagged with a non-sequential His tag (HAT-b-2-m), the fusion protein binds equally well to the immobilizer Ni-Chelate plate. Using the competitor plates the HAT-b-2-m does not bind as well as the His-b-2-m.

Acknowledgements

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Credits

Assay and protocol for Ni-Chelate plate preparation was designed by Eva Jauho and Maria Nielsen.

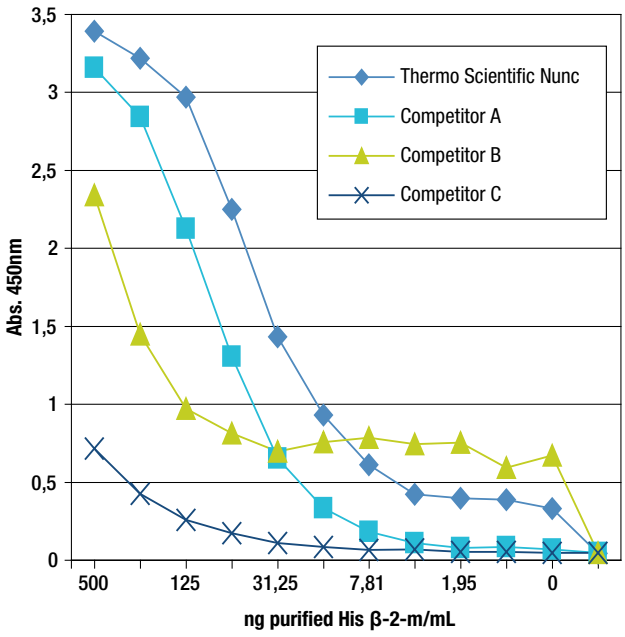

Fig. 2.

Detection of a purified His-tagged fusion protein (purified His-ß-2-m, preparation B). The His-tagged fusion protein was applied in a 1:2 dilution to the plates. As a negative control, buffer without His-tagged fusion protein was applied.

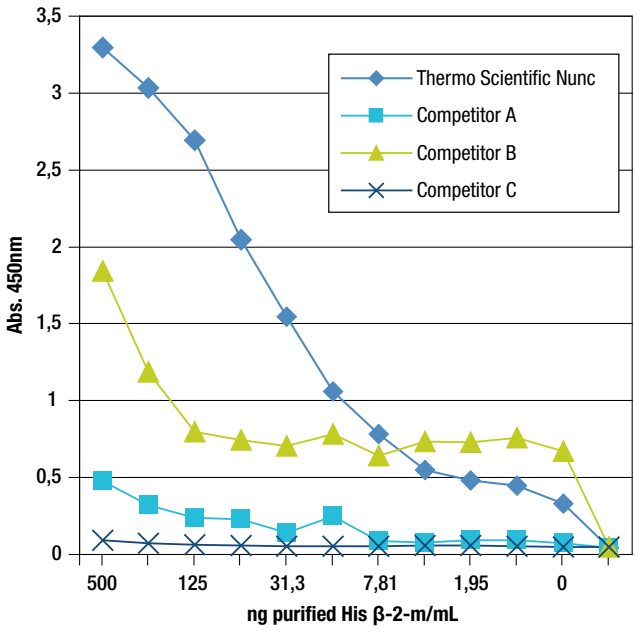

Fig. 3.

Detection of the fusion protein tagged with an non-sequential histidine tag (purified HAT-ß-2-m, preparation C). The purified HAT-ß-2-m was added in a 1:2 dilution to the plates. As a negative control, buffer without His-tagged fusion protein was added.

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