Thermo Scientific Nunc Immobilizer Nickel-Chelate

Application Note

Key Words

Thermo Scientific™ Nunc™ Immobilizer™ Ni-Chelate plates, Ni-Chelate, His-tagged proteins, recombinant fusion proteins, low detection limit.

Goal

The goal of this application note is to show the optimal protocol for the Nunc Immobilizer Ni-Chelate 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. In addtion to show that the assay time can be reduced due to fewer steps in the assay.

Nunc Immobilizer Ni-Chelate plate is manufactured using a patented photochemical method[1] for covalent coupling of ligands to polymer surfaces. A Ni-Chelate complex is coupled via a spacer to the plates.

The Nunc Immobilizer Ni-Chelate plates are designed for optimal binding of fusion proteins that have been tagged with an amino acid sequence containing six consecutive histidine (His) molecules.

Recommended coupling protocol

Materials

- Nunc Immobilizer Ni-Chelate plates
- Coupling buffer: 0.01 M KCl
- Washing buffer: PBST (Phosphate Buffered Saline (pH 7.3) containing 0.05% (v/v) Tween 20).

Protocol for 96 well plates

1. Pre-wash the plates with PBST (3 x 300 µL/well).
2. Prepare a solution of a 6 x His-tagged protein (0.01-1 µg/mL) in 0.01 M KCl.
3. Add the protein solution to the wells of the Nunc Immobilizer Ni-Chelate plate (100 µL/well).
4. Incubate the plate with gentle agitation (100 rpm) at 20-25°C for two hours or at +4°C overnight.
5. Aspirate the wells and wash with PBST (3 x 300 µL).

Protocol for 384 well plates

1. Pre-wash the plate with PBST (3 x 100 µL/well).
2. Prepare a solution of a His-tagged protein (0.01-1 µg/mL) in 0.01 M KCl.
3. Add the protein solution to the wells of the Nunc Immobilizer Ni-Chelate plate (50 µL/well).
4. Incubate the plate with gentle agitation (100 rpm) at 20-25°C for two hours or at +4°C overnight.
5. Aspirate the wells and wash with PBST (3 x 100 µL).

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We recommend the inclusion of 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. 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 His-tagged fusion protein

The performance of the Immobilizer Ni-Chelate surface is illustrated using the transparent 96 well plate.

A His-tagged fusion protein (25 kDa) is applied to the plate in a series of dilutions. The amount of immobilized His-tagged fusion protein is detected by addition of an antibody to the fusion protein. The antibody is conjugated to horseradish peroxidase (HRP). The amount of bound 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 immobilized 6 x His-tagged fusion protein.

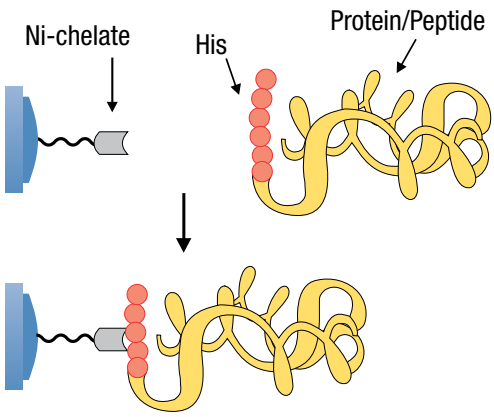

Fig. 1.

Coupling of a His-tagged protein/peptide to the Immobilizer Ni-Chelate plate.

The following assay was performed on the Immobilizer Ni-Chelate plate.

His-tagged fusion protein determination

1. Pre-wash the plate with PBST (3 x 300 µL/well).
2. Prepare a series of His-tagged fusion protein solutions in 0.01 M KCl (0.001-0.5 µg/mL).
3. Dispense 100 µL of each of these solutions into the wells of a transparent Nunc Immobilizer Ni-Chelate plate in rows 1-10. Add 0.01 M KCl to rows 11-12 as a negative control.
4. Incubate the plate with gentle agitation (100 rpm) at 20-25°C for two hours.
5. Aspirate and wash the plate with PBST (3 x 300 µL).
6. Dispense 100 µL of an antibody to the fusion protein diluted in PBST to the wells of rows 1-11 on the plate. PBST is added to row 12 as a blank value.
7. Incubate the plate with gentle agitation (100 rpm) at 20-25°C for one hour.
8. Aspirate the wells and wash with PBST (3 x 300 µL).
9. Incubate the plate with gentle agitation (100 rpm) at 20-25°C 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 stop solution to the plate (100 µL/well).
14. Read the absorbance at 450 nm.

As can be seen from Fig. 2, the background is extremely low when using the Immobilizer Ni-Chelate plate. This results in a high signal to noise ratio and a low detection limit, which in this assay is 15 ng His-tagged fusion protein per mL (1.5 ng/well) when assuming OD=0.5 as a significant response relative to the background.

Furthermore, the assay time is quite short on the Immobilizer Ni-Chelate plate for detection of His-tagged fusion proteins. This is due to the reduced number of incubation steps in the assay.

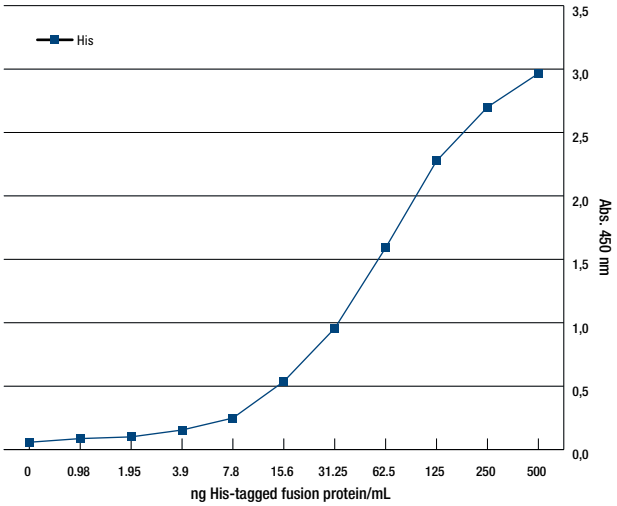

Fig. 2.
Dilution of a His-tagged fusion protein (25 kDa) in 0.01 M KCl.

Acknowledgements

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Credits

Assay and protocol for Ni-Chelate plate preparation was designed by Eva Jauho and Knud Lerstrup.

References

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