Application Note

Thermo Scientific Nunc Immobilizer Streptavidin and Amino

Key Words

Thermo Scientific™ Nunc™ Immobilizer™ plates and strips, covalent binding, affinity binding surface, Immobilizer Amino, Immobilizer Streptavidin, ELISA, biotinylated capture molecule, binding of peptides, binding of nucleotides.

Goal

The goal of this application note is to show that the characteristics of Immobilizer plates and strips are highly suitable for many applications. Further to show that small ligands are able to bind with a high signal to noise background, low detection limit, no blocking and short incubation time.

Excellent binding capacity

A unique surface chemistry has been created for the Thermo Scientific Nunc Immobilizer plates and strips which provides excellent covalent coupling for optimal target molecule orientation and stability.

The concentrations of target molecule required for coupling is usually very low compared with what is needed for passive binding. The plate surface provides a high affinity surface especially for the coupling of small ligands.

High signal to noise ratio

Assays conducted on the Nunc Immobilizer plates and strips are characterized by high signal readouts (Fig. 1).

This, in combination with no or very low non-specific binding and thus negligible background noise, ensures superior signal to noise ratios (Fig. 2).

Highly reproducible results

Nunc Immobilizer plates and strips are manufactured for consistent and reproducible assay results. The covalently bound monolayer coating of the plates is highly uniform. Leaching is minimal, even when using very stringent washing steps. These characteristics make the Immobilizer plates and strips highly suitable for many applications.

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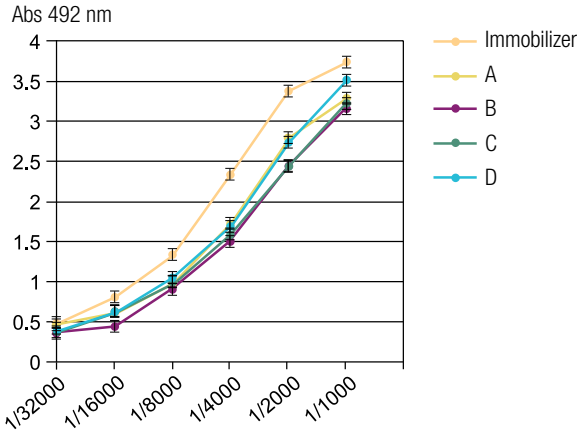


Fig. 1.

Immobilizer Streptavidin plates were compared with 4 other suppliers (A, B, C, and D) of streptavidin plates, in an ELISA. Low concentrations of biotinylated anti-human IgG, (in 6 concentrations) was coupled to the streptavidin surface. Following addition of human IgG, the amount of bound human IgG was detected by adding HRP conjugated anti-human IgG and visualized by incubation with OPD substrate and measuring at 492 nm. The result shows that the required amount of added human IgG appeared to be approx. half of the amount that is required for the other suppliers' plates when measured at an OD of 1.

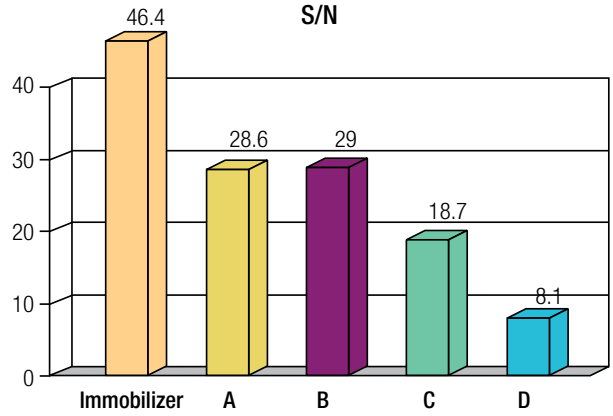
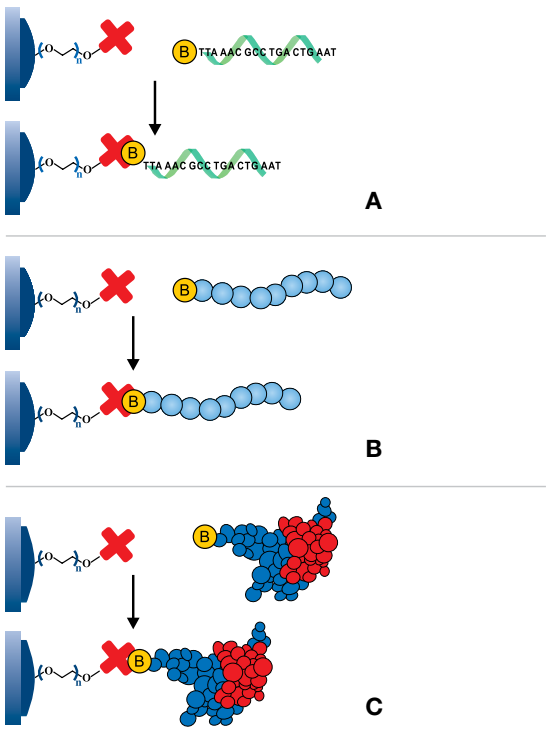


Fig. 2.

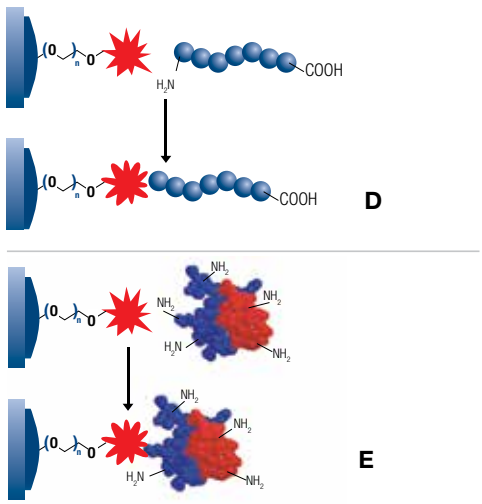
The signal to noise ratio was obtained on streptavidin plates from 4 different suppliers (A, B, C, and D). They were compared to the signal to noise ratio obtained on Immobilizer Streptavidin plates in an assay, where a biotinylated 15-mer DNA oligonucleotide bound to the streptavidin, was hybridized to a 98 bp DIG-incorporated pUC19 amplicon (400 fmol/well). The amount of hybridized DNA was detected by adding an anti-DIG antibody conjugated to HRP and reading of the signal at 450 nm following addition of TMB. The noise signal was obtained from all plates by following an identical procedure, but replacing the pUC19 plasmid template with water.

Mechanism of action Immobilizer Streptavidin



Coupling of A) biotinylated DNA oligonucleotide, B) biotinylated peptide, and C) biotinylated protein to the streptavidin conjugated photoprobes on the solid phase of the Streptavidin Immobilizer plates.

Mechanism of action Immobilizer Amino



Coupling of D, peptides or E, proteins to the Immobilizer Amino surface.

No blocking

In contrast to most other coated plates, the Immobilizer plates and strips do not require separate costly, labor intensive and time-consuming blocking steps to prevent non-specific binding.

This is also due in part to the placement and density of the active sites and their spacer arms. These create a "molecular shield" which prevents direct access to the polystyrene hydrophobic surface, where non-specific binding could otherwise take place.

Higher signal using low sample concentration

The Immobilizer products are characterized by the high signals obtained even with very low sample concentrations.

As illustrated in Fig. 3, in certain cases when samples at one tenth concentration are used, the signals obtained on the Immobilizer surface are greater than those obtained using normal high binding ELISA plates and passive absorption.

Short incubation time

Coupling of target molecules to the Immobilizer surface takes place very quickly, compared to the time required for stable coating by passive adsorption.

The Immobilizer surface can be saturated after approximately 4 hours incubation with gentle shaking.

The coupling efficiency is typically unaffected by pH. However, one must be aware that pH can conformationally change the biomolecules that are bound. The presence of non-ionic detergents suppresses the coupling reaction.

Long term stable at room temperature

The shelf life of the Immobilizer plates and strips exceeds one year, when they are stored in their unopened packaging at room temperature. The manufacturing date is printed on the package. The Immobilizer Streptavidin has a minimum shelf life post manufacturing of 18 months. For Immobilizer Amino surface the period is at least 2 years.

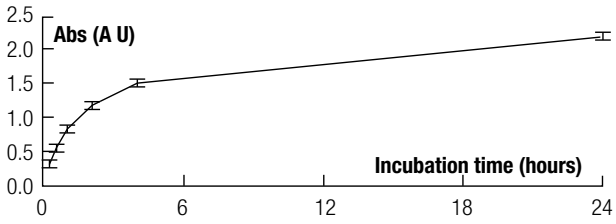


Fig. 4.

Effect of incubation time on peptide coupling

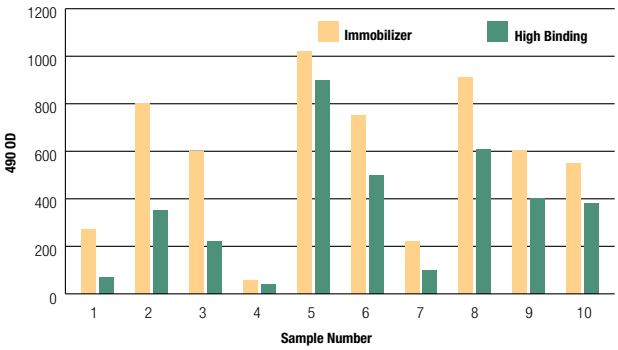


Fig. 3.

A 40 amino acid peptide from hepatitis virus C was covalently coupled to Immobilizer Amino plate or passively coated on a High Binding ELISA plate. A peptide concentration of 20 µg/mL was used for coating the High Binding ELISA plate while a peptide concentration of 2 µg/mL was used with the Immobilizer Amino plate. The plates were developed by incubating a dilution of human serum washing, and then adding an enzyme conjugated anti-IgG reagent. Result: The Immobilizer Amino plate produced higher signals using 1/10 the amount of peptide.

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