Application Note

Thermo Scientific Nunc Immobilizer Streptavidin

Application example: Colorimetric detection of human IgG in blood plasma samples

Key Words

Immobilizer, Streptavidin, polystyrene surface, Streptavidin plates, sandwich ELISA.

Goal

The goal of this tech note is to perform a sandwich immunoassay using Thermo Scientific™ Nunc™ Immobilizer™ Streptavidin plates. Further to show that the plates provide a flexible and sensitive immunoassay platform.

This note describes a sandwich immunoassay performed using Nunc Immobilizer Streptavidin plates. These plates are manufactured using a patented photochemical method[1] for covalent coupling of ligands to polymer surfaces.

Streptavidin is a high affinity biotin-binding protein, isolated from Streptomyces avidinii. It is covalently coupled to the polystyrene surface via a polyethylene glycol spacer arm.

Nunc Immobilizer Streptavidin plates are optimized for easy and sensitive detection of various types of biotinylated biomolecules such as oligonucleotides, peptides, and proteins.

Introduction

The assay was performed in a clear 96 well Nunc Immobilizer Streptavidin plate to demonstrate a non-competitive sandwich ELISA. Using biotinylated capture antibody and horseradish peroxidase (HRP) labeled detector antibody, human IgG in plasma samples was detected.

In this type of assay, the amount of bound detector antibody is proportional to the amount of analyte present in the sample. Bound HRP detector antibody is measured by incubating the wells with the substrate OPD and reading the resulting absorbance.

Materials

- 96 Well Nunc Immobilizer Streptavidin plates
- PBST, pH 7.2 (Phosphate buffered saline containing 0.05% Tween 20)
- Biotinylated rabbit and human IgG
- HRP labeled anti-human IgG
- Thermo Scientific™ Nunc™ MiniSorp™ Tubes
- Ortho-phenyl-diamine
- H_2O_2
- H_2SO_4 0.5 M

Protocol

1. Pre-wash the plate with 3 x 300 µL/well PBST buffer.
2. Dilute biotinylated rabbit antihuman IgG 1:8000 in PBST buffer. Add 100 µL/well of the solution to the plate. Note: this dilution should be performed in a low-protein binding plastic tube (e.g. Nunc MiniSorp).
3. Incubate the plate with gentle agitation for 1 hour at room temperature.
4. Aspirate the plate and wash three times with PBST buffer (3 x 300 µL/well).

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5. Make a 1:2 titration of your human plasma sample. Prefill the appropriate wells with 100 µL of PBST. Starting with a 1:100 dilution of plasma in PBST, serially transfer 100 µL.
6. Incubate the plate with gentle agitation for 1 hour at room temperature.
7. Aspirate the plate and wash three times with PBST buffer (3 x 300 µL/well).
8. Prepare a 1:2000 dilution of HRP labeled secondary antibody in PBST buffer. Add 100 µL/well to the plate. Note: this dilution should be performed in a low-protein binding plastic tube (e.g. Nunc MiniSorp).
9. Incubate the plate with gentle agitation for 1 hour at room temperature.
10. Aspirate the plate and wash three times with PBST buffer (3 x 300 µL/well).
11. Prepare a solution of orthophenylene-diamine (OPD), 6 mm; and H_2O_2, 4 mm in 100 mm citric acid buffer pH 5.0. Add 100 µL/well to the plate and incubate in the dark for 10 minutes at room temperature.
12. The enzyme reaction is stopped with H_2SO_4, 0.5 M (100 µL/well) and the absorbance is measured at 492 nm with an ELISA reader.

Summary

The results show (Fig. 1) that an analyte, in this case human IgG, can be successfully and easily quantitated via a sandwich immunoassay that employs a biotinylated capture antibody and a streptavidin coated plate. In our example, the signal is proportional to the amount of plasma added to the well. The controls indicate that the signal is specific.

This assay is easy to perform. The plates were coated rapidly (1 hour), and a low background was observed even though no specific blocking step was employed. Nunc Immobilizer Streptavidin plates provide a flexible and sensitive immunoassay platform.

Specifications: Clear 96 well plates

- Streptavidin coated area; 100 µL/well
- Total binding capacity for biotin, 5 ng/well (20 pmol/well)*
- Stable at room temperature for 18 months after manufacturing
- Coefficient of variation (CV) < 5% well-to-well

* The binding capacity may vary depending on the size and steric properties of the biotinylated biomolecule being used.

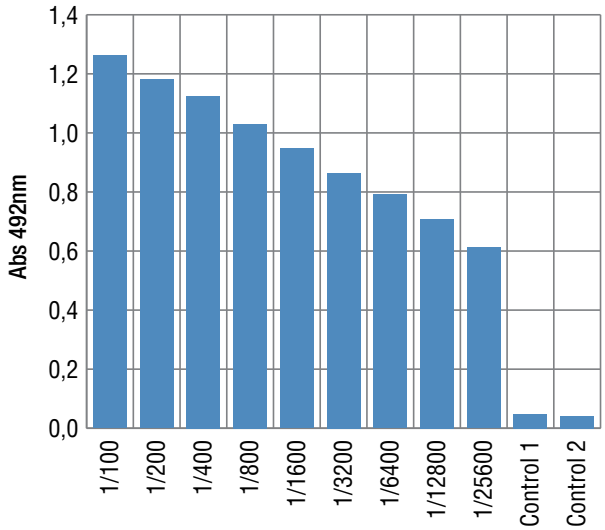
References

1. Koch T, Jacobsen N, Fensholdt J, Boas U, Fenger M, Jakobsen MH.
Photochemical Immobilization of Anthraquinone Conjugated oligonucleoides and PCR Amplicons on solid Surfaces.
Bioconjugate Chem. 11 (2000), 474-483.

Credits

Assay and protocol for Streptavidin plate preparation was designed by Kirsten Gerner-Smidt.

Fig. 1 Measurement of Human IgG on Immobilizer Streptavidin plate



Control 1 without biotinylated antibody.

Control 2 without human plasma.

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