APPLICATION NOTE

Nunc Immobilizer Streptavidin

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Key Words: Nunc Immobilizer Streptavidin Surface, Affinity attachment, ELISA, biotinylated bio-molecules, Protocol.

Goal

The goal of this protocol is to outline the specific steps which are necessary in order to be successful when using the Immobilizer Streptavidin surface for binding of biotinylated bio-molecules.

Introduction

The Nunc™ Immobilizer® Streptavidin Plates/Strips are manufactured using a patented photochemical method[1] for covalent coupling of ligands to polymer surfaces. Streptavidin, a high affinity biotin-binding protein isolated from Streptomycetes avidinii, is covalently coupled via a spacer to the plastic plates/strips. Streptavidin has a molecular weight of approx. 60,000 Dalton and has an isoelectric point (pI) close to 5. The Nunc Immobilizer Streptavidin Plates/Strips are designed and optimized for detection of various types of biotinylated bio-molecules like biotinylated oligo-nucleotides, peptides and proteins.

Materials

Nunc Immobilizer Streptavidin Plates/Strips Biotinylated molecule of choice

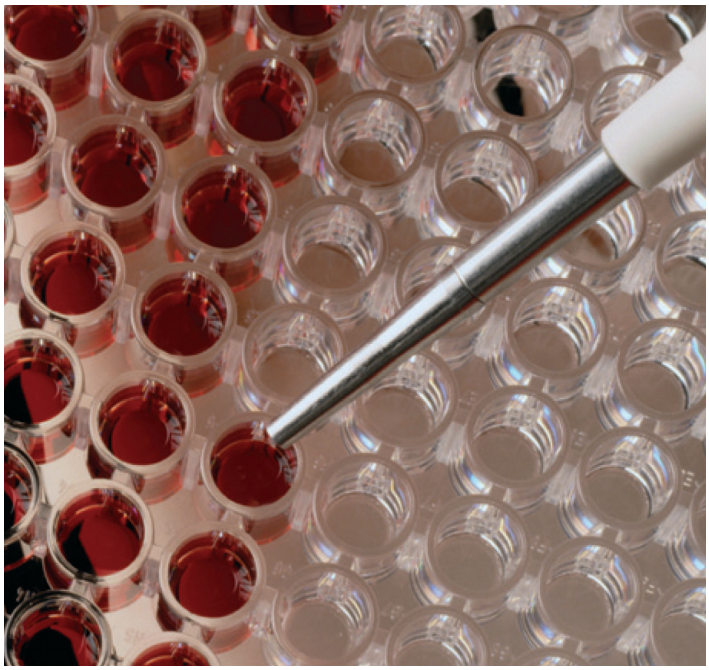
Reagents

5 x SSCT, pH 7.0 (5 x SSC (750 mM NaCl, and 75 mM Sodium Citrate) containing 0.05% (v/v) TWEEN® 20)

2 x SSCT, pH 7.0 (2 x SSC (300 mM NaCl, and 30 mM Sodium Citrate) containing 0.05% (v/v) TWEEN 20)

PBST, pH 7.2 (Phosphate Buffered Saline containing 0.05% (v/v) TWEEN 20)

Note: Buffers should be used within one week from preparation.



Recommended coupling concentrations Biotinylated oligonucleotides:

We recommend using the following concentration range: 0.01 – 0.5 µM diluted in 5 x SSCT buffer pH 7.0

Biotinylated peptides: We recommend using the following concentration range: 1 ng/ml – 1 µg/ml diluted in a PBST buffer pH 7.2

Biotinylated proteins: We recommend using the following concentration range: 0.05 µg/ml – 5 µg/ml diluted in a PBST buffer pH 7.2

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Coupling protocol for 96 well plate, and 8 well strips:

1. Pre-wash your plate with 3 x 300 µl/well PBST or 5 x SSCT buffer without any incubation step. This is done to ensure improved readouts and a very low coefficient of variation (CV%)
2. Prepare a solution of your biotinylated molecule in PBST buffer or 5 x SSCT (oligonucleotides)
3. Add the solution to the wells of a Nunc Immobilizer Streptavidin plate/strip (100 µl/well)
4. Incubate the plate with gentle agitation for 1 hour at room temperature (20° - 25°C)
5. Aspirate the wells and wash with PBST or 2 x SSCT (oligonucleotides) 3 x 300 µl/well
6. Your surface is now ready for assay application

Coupling protocol for 384 well plate:

1. Pre-wash your plate with 3 x 100 µl/well PBST or 5 x SSCT buffer without any incubation step. This is done to ensure improved readouts and a very low coefficient of variation (CV%)
2. Prepare a solution of your biotinylated molecule in PBST buffer or 5 x SSCT buffer (oligonucleotides)
3. Add the solution to the wells of a Nunc Immobilizer Streptavidin plate (50 µl/well)
4. Incubate the plate with gentle agitation for one hour at room temperature (20° - 25°C)
5. Aspirate the wells and wash with PBST or 2 x SSCT (oligonucleotides) 3 x 100 µl/well
6. Your surface is now ready for assay application

Specifications

Streptavidin coated area ~100 µl/well (96 well format)
Stable when stored at room temperature to the expiration date, which appears on the case label

Trademarks and patents

TWEEN 20 is a registered trademark of ICI American Inc., U.S.A. Immobilizer is a trademark of Exiqon A/S, Vedbaek, Denmark.

The product is produced under license from Exiqon A/S - EP 08 20483 and foreign applications and patents.

References:

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

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