INSTRUCTIONS

Pierce® Streptavidin Coated 96-Well Plates

0463.3

Number	Description
15120	**Pierce Streptavidin Coated Plate (clear, 8-well strips),** 5 plates
15122	**Pierce Streptavidin Coated Plate (clear, 8-well strips),** 5 × 5 plates
15124	**Pierce Streptavidin Coated Plate (clear, 96-well),** 5 plates
15126	**Pierce Streptavidin Coated Plate (clear, 96-well),** 5 × 5 plates
15118	**Pierce Streptavidin Coated Plate (white, 96-well),** 5 plates
15119	**Pierce Streptavidin Coated Plate (black, 96-well),** 5 plates Blocking Buffer: These plates are supplied blocked with SuperBlock® Blocking Buffer Binding Capacity: ~5pmol D-biotin/well Activation Level: 100µL
15121	**Pierce Streptavidin Coated Plate (clear, 8-well strips),** 5 plates
15125	**Pierce Streptavidin Coated Plate (clear, 96-well),** 5 plates
15218	**Pierce Streptavidin Coated Plate (white, 96-well),** 5 plates
15219	**Pierce Streptavidin Coated Plate (black, 96-well),** 5 plates Blocking Buffer: These plates are supplied blocked with Blocker™ BSA Binding Capacity: ~10pmol D-biotin/well Activation Level: 200µL

Storage: Upon receipt store plates at 4°C in unopened pouches. Once opened, place unused plates in a resealable bag with desiccant and store at 4°C. Plates are shipped at ambient temperature.

Introduction

The Thermo Scientific Pierce Streptavidin Coated Plates are made of polystyrene and are ideal for binding assays using biotinylated molecules. These plates are especially advantageous when direct adsorption to polystyrene plates denatures antibodies or the target molecule. Streptavidin has no carbohydrate groups and an isoelectric point of 5-6, resulting in low nonspecific interactions. The streptavidin coated plates are available in clear for colorimetric assays, white for chemiluminescent assays, and black for fluorescent assays.

Example ELISA Procedure

The following protocol describes a generalized enzyme-linked immunosorbent assay using a biotinylated capture antibody. Please see the reference list for other possible applications using streptavidin-coated microplates.

A. Materials Required

- Wash Buffer: Tris-buffered saline (25mM Tris, 150mM NaCl; pH 7.2; Product No. 28376), 0.1% BSA, 0.05% Tween®-20 Detergent; alternatively, use Thermo Scientific Blocker BSA (Product No. 37520) supplemented with 0.05% Tween-20
- Biotinylated capture antibody adjusted to 10µg/mL, or other appropriate concentration, with Wash Buffer
- Antigen adjusted to appropriate concentration with Wash Buffer

- Primary antibody adjusted to appropriate concentration with Wash Buffer
- Enzyme-labeled secondary antibody adjusted to appropriate concentration with Wash Buffer
- Appropriate enzyme substrate: example substrates are the TMB Substrate Kit (Product No. 34021) for horseradish peroxidase and the Phosphatase Substrate Kit (Product No. 37620) for alkaline phosphatase

B. Method

1. Wash each well three times with 200µL of Wash Buffer. Add 100µL of the biotinylated capture antibody to each well and incubate for 2 hours with shaking at room temperature.
2. Wash each well three times with 200µL of Wash Buffer. Make a serial dilution of the antigen and add 100µL to each well. Incubate plate for 30 minutes with shaking at room temperature.
3. Wash each well three times with 200µL of Wash Buffer. Add 100µL of the primary antibody to each well and incubate plate for 30 minutes at room temperature.
4. Wash each well three times with 200µL of Wash Buffer. Add 100µL of the enzyme-labeled secondary antibody to each well. Incubate plate for 30 minutes with shaking at room temperature.
5. Wash each well three times with 200µL of Wash Buffer.
6. Follow the manufacturer's instructions for the specific detection system.

Procedure for Determining Binding Activity of the Coated Plates

The binding activity of the plates can be tested using Thermo Scientific Biotinylated Alkaline Phosphatase (Product No. 29339) and PNPP (Product No. 37620) or Biotinylated Horseradish Peroxidase (Product No. 29139) and TMB (Product No. 34021).

1. Rinse each well with three times with 200µL of wash buffer (e.g., TBS).
2. Prepare a 1mg/mL solution of the biotinylated enzyme. Make 1:2 serial dilutions using a 1:1000 dilution for the first well. Incubate the wells for 1 hour at room temperature.
3. Wash each well three times with 200µL of TBS containing 0.05%Tween-20.
4. Incubate with 100µL of substrate solution for 15 minutes at room temperature.
5. Measure the absorbance of each well. Active plates result in an absorbance of 0.5 to 1.0 OD at 405nm.

Related Thermo Scientific Products

37070	**SuperSignal® ELISA Pico Chemiluminescent Substrate,** 100mL
15169	**QuantaBlu™ Fluorogenic Peroxidase Substrate Kit**
34028	**1-Step™ Ultra TMB-ELISA,** 250mL
37621	**1-Step PNPP,** 100mL
29339	**Biotinylated Alkaline Phosphatase,** 1mg
29139	**Biotinylated Horseradish Peroxidase,** 5mg
15075	**Reagent Reservoirs,** 200/pkg
15082	**Microtube Racked System,** 960 tubes
15036	**Sealing Tape for 96-Well Plates,** 100/pkg
45360	**Pierce Streptavidin Coated Plate Immunoprecipitation Kit**
21425	**EZ-Link® Sulfo-NHS-Biotinylation Kit**
21335	**EZ-Link Sulfo-NHS-LC-Biotin,** 100mg

General References

Adler, K., *et al.* (1992). Rapid DNA detection using a fluorescein-anti-fluorescein-based (FAF) ELOSA. *Biotech Update (Du Pont)* 160-4.

Butler, J.E., *et al*. (1992). The physical and functional behavior of capture antibodies adsorbed on polystyrene. *J Immunol Meth* **150:**77-90.

Chaiet, I, and Wolf, F.J. (1974). The properties of streptavidin, a biotin-binding protein produced by Streptomycetes. *Arch Biochem Biophys* **106:**1-5.

Estrada, G. *et al.* (1996). Sequence-specific detection of PCR-amplified DNA by restriction enzyme release of hybrids. *Mole Cell Probes* **10:**179-85.

Ferre-Aubineau, V., *et al*. (1995). Colorimetric microwell plate hybridization assay using monoclonal antibody for detection of an amplified human immunodeficiency virus target. *J Virol Methods* **55:**145-51.

Hobson, D., *et al.* (2003). *In situ* transduction of target cells on solid surfaces by immobilized viral vectors. *BMC Biotech.* **3:**4. This article is available from: http://www.biomedcentral.com/1472-6750/3/4

Moroder, L., *et al.* (1992). Induction and detection of anti-peptide antibody specificity is critically affected by the mode of hapten presentation. *Biol Chem Hoppe-Seyler* **373:**315-21.

Wilbur, S., *et al.* (1988). Biotin regents for antibody pretargeting. 3. Synthesis, radioiodination, and evaluation of biotinylated starburst dendrimers. *Bioconjugate Chem* **9:**813-25.

Wilbur, S., *et al.* (1997). Biotin reagents for antibody pretargeting. 2. Synthesis and *in vitro* evaluation of biotin dimers and trimers for cross-linking of streptavidin. *Bioconjugate Chem* **8:**819-32.

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