# **INSTRUCTIONS**

# Pierce<sup>®</sup> Streptavidin Coated 384-Well Plates

# 15405 15406 15407

Number	Description
15405	Pierce Streptavidin Coated Plate (clear, 384-well), 5 each
15406	Pierce Streptavidin Coated Plate (white, 384-well), 5 each
15407	Pierce Streptavidin Coated Plate (black, 384-well), 5 each
	Blocking Buffer: These plates are supplied blocked with SuperBlock <sup>®</sup> Blocking Buffer
	Binding Capacity: ~4pmol D-biotin/well
	Coating Volume: 50µL
	Blocking Volume: 100µL

**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.

## 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 Pierce Streptavidin Coated Plates are available in clear for colorimetric assays, white for chemiluminescent assays, and black for fluorescent assays.

# Example ELISA Protocol using Streptavidin Coated Plates

### A. Materials Required

- Wash Buffer: Tris-buffered saline (25 mM Tris, 150 mM NaCl; pH 7.2; Product No. 28376), 0.1% BSA, 0.05% Tween<sup>®</sup>-20 Detergent; alternatively, use Thermo Scientific Blocker BSA (Product No. 37520) supplemented with 0.05% Tween-20 Detergent
- 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 Thermo Scientific 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  $100\mu$ L of Wash Buffer. Add  $50\mu$ L of the biotinylated capture antibody to each well and incubate for 2 hours at room temperature.
- 2. Wash each well three times with 100µL of Wash Buffer. Make a serial dilution of the antigen and add 50µL to each well. Incubate plate for 30 minutes at room temperature.
- 3. Wash each well three times with 100μL of Wash Buffer. Add 50μL of the primary antibody to each well and incubate plate for 30 minutes at room temperature.
- Wash each well three times with 100μL of Wash Buffer. Add 50μL of the enzyme-labeled secondary antibody to each well. Incubate plate for 30 minutes at room temperature.



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- 5. Wash each well three times with 100µL of Wash Buffer.
- 6. Follow the manufacturer's instructions for the specific detection system.

## **Related Thermo Scientific Products**

37070	SuperSignal <sup>®</sup> ELISA Pico Chemiluminescent Substrate, 100mL, peroxidase substrate
15169	QuantaBlu™ Fluorogenic Peroxidase Substrate Kit
34028	1-Step <sup>™</sup> Ultra TMB-ELISA, 250mL, colorimetric peroxidase substrate
37621	1-Step PNPP, 100mL, colorimetric phosphatase substrate
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
15511	Pierce NeutrAvidin High Binding Capacity Coated Plates (clear, 384-well), 5 each
15512	Pierce NeutrAvidin High Binding Capacity Coated Plates (white, 384-well), 5 each
15513	Pierce NeutrAvidin High Binding Capacity Coated Plates (black, 384-well), 5 each

#### **General References**

Denlinger, L.C., *et al.* (2001). Cutting Edge: The nucleotide receptor P2X7 contains multiple protein- and lipid-interaction motifs including a potential binding site for bacterial lipopolysaccharide. *J Immunol* **167**:1871-6.

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

Hiller, Y. et al. (1987). Biotin binding to avidin. Oligosaccharide side chain not required for ligand association. Biochem J 248:167-71.

Holmstrom, K., *et al.* (1993). A highly sensitive and fast non-radioactive method for detection of polymerase chain reaction products. *Anal Biochem* **209**:278-283.

Simon, M.D., et al. (2004). A phage display selection of engrailed homeodomain mutants and the importance of residue Q50. Nucl Acid Res 32(12):3623-31.

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