INSTRUCTIONS

Pierce® High Sensitivity Streptavidin Coated Plates

15520 15525

2234.0

Number	Description
15520	**Pierce High Sensitivity Streptavidin Coated Plates** (clear, 12 × 8-well strips), 5 plates
15525	**Pierce High Sensitivity Streptavidin Coated Plates** (black, 12 × 8-well strips), 5 plates

Detection range: 5-300 ng/ml (100 µl) of biotinylated IgG

Blocking Buffer: These plates are supplied blocked with Blocker™ BSA

Activation Level: 250 µl

Storage: Upon receipt store at 4°C. Product is shipped at ambient temperature.

Introduction

The Thermo Scientific Pierce High Sensitivity Streptavidin Coated Plates are ideal for assaying low-abundance biotinylated molecules such as biotinylated IgG. 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 High Sensitivity Streptavidin Coated Plates are available in clear for colorimetric assays and black for fluorescent and chemiluminescent assays.

Important Product Information

- The detection range (5-300 ng) was determined using 100 µl of biotinylated rabbit IgG, goat anti-rabbit conjugated to horseradish peroxidase (100 ng/ml) and Thermo Scientific QuantaBlu Fluorogenic Peroxidase Substrate (Product No. 15169).
- For best results, use the Pierce High Sensitivity Plates for biotinylated molecules that are > 26 kDa. To obtain high-capacity binding of biotinylated molecules or for binding small molecules such as biotinylated peptides or oligonucleotides (for hybridization assays), use Pierce High Binding Capacity Streptavidin Coated Plates (Product No. 15500 or 15503).
- Once opened, place unused plates in a resealable bag with desiccant and store at 4°C.

Example ELISA Procedure

The following protocol describes a general enzyme-linked immunosorbent assay using a biotinylated capture antibody. See references for other possible applications.

A. Materials Required

- Phosphate-buffered saline (PBS; 25 mM phosphate, 150 mM NaCl; pH 7.2; Product No. 28372)
- Wash Buffer: PBS with 0.05% Tween®-20 Detergent
- Biotinylated capture antibody adjusted to the appropriate concentration (e.g., 50 ng/ml) with PBS
- Antigen adjusted to the appropriate concentration with PBS
- Primary antibody adjusted to the appropriate concentration with PBS
- Enzyme-labeled secondary antibody adjusted to the appropriate concentration with Wash Buffer
- Enzyme substrate such as QuantaBlu™ Fluorogenic Peroxidase Substrate or TMB Substrate Kit (Product No. 34021) for horseradish peroxidase, or the Phosphatase Substrate Kit (Product No. 37620) for alkaline phosphatase

B. Method

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

Related Thermo Scientific Products

37070	**SuperSignal® ELISA Pico Chemiluminescent Substrate** for HRP, 100 ml
15169	**QuantaBlu Fluorogenic Peroxidase Substrate Kit**
15159	**QuantaRed Enhanced Chemifluorescent HRP Substrate**
34028	**1-Step™ Ultra TMB-ELISA** for HRP, 250 ml
37621	**1-Step PNPP** colorimetric AP substrate, 100 ml
29339	**Biotinylated Alkaline Phosphatase,** 1 mg
29139	**Biotinylated Horseradish Peroxidase,** 5 mg

References

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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:**278-83.

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.