INSTRUCTIONS

Pierce® NeutrAvidin® High Binding Capacity Coated 96-Well Plates

15507 15508 15509 15510 0922.2

Number	Description
15507	**Pierce NeutrAvidin High Binding Capacity Coated Plates (clear 96-well),** 5 each
15508	**Pierce NeutrAvidin High Binding Capacity Coated Plates (clear 8-well strips),** 5 each
15509	**Pierce NeutrAvidin High Binding Capacity Coated Plates (white 96-well),** 5 each
15510	**Pierce NeutrAvidin High Binding Capacity Coated Plates (black 96-well),** 5 each
	Blocking Buffer: Supplied blocked with SuperBlock® Blocking Buffer
	Binding Capacity: ~60pmol D-biotin/well
	Coating Volume: 100µL
	Blocking Volume: 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.

Introduction

The Thermo Scientific Pierce NeutrAvidin Coated Plates are ideal for capturing biotin-labeled molecules without interference from nonspecific binding. NeutrAvidin Protein is deglycosylated avidin, which reduces lectin binding to undetectable levels while retaining stability and biotin-binding affinity. NeutrAvidin Protein offers the advantages of a near-neutral pI (6.3), to minimize nonspecific adsorption, and the lack of the RYD sequence, which eliminates nonspecific binding to the RGD binding domain of adhesion receptors present in a variety of cells. NeutrAvidin Protein yields the lowest nonspecific binding among the known biotin-binding proteins. The clear, white and black plates can be used with colorimetric, chemiluminescent and fluorescent detection methods, respectively.

Our patented coating technology is used to create high binding capacity (HBC) Pierce NeutrAvidin Coated Plates. These HBC plates provide a wider detection range and better curve linearity for small biotinylated ligands, such as peptides and oligonucleotides, than the standard coated plates.

Example ELISA Protocol using Pierce NeutrAvidin Coated Plates

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 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 200µL of Wash Buffer. Add 100µL of the biotinylated capture antibody to each well and incubate for 2 hours 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 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 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.

Related Thermo Scientific Products

37070	**SuperSignal® ELISA Pico Chemiluminescent Substrate,** 100mL, peroxidase substrate
15169	**QuantaBlu™ Fluorogenic Peroxidase Substrate Kit**
34028	**1-Step™ 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.