INSTRUCTIONS

Pierce® NeutrAvidin® Coated 96-Well Plates

0611.4

Number	Description
15127	**Pierce NeutrAvidin Coated Plates (clear, 8-well strips),** 5 each
15129	**Pierce NeutrAvidin Coated Plates (clear, 96-well),** 5 each
15116	**Pierce NeutrAvidin Coated Plates (white, 96-well),** 5 each
15117	**Pierce NeutrAvidin Coated Plates (black, 96-well),** 5 each Activation Level: 100µL Binding Capacity: ~15pmol D-biotin/well Blocking Buffer: These plates are supplied blocked with SuperBlock® Blocking Buffer
15128	**Pierce NeutrAvidin Coated Plates (clear, 8-well strips),** 5 each
15123	**Pierce NeutrAvidin Coated Plates (clear, 96-well),** 5 each
15216	**Pierce NeutrAvidin Coated Plates (white, 96-well),** 5 each
15217	**Pierce NeutrAvidin Coated Plates (black, 96-well),** 5 each Activation Level: 200µL Binding Capacity: > 15pmol D-biotin/well Blocking Buffer: These plates are supplied blocked with Blocker™ BSA

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 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.

Example ELISA Protocol using 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.

Procedure for Determining Binding Activity of the NeutrAvidin Coated Plates

The binding activity of the plates may 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 Detergent.
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

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:**278-83.

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.

This product ("Product") is warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation") and to be free from defects in material and workmanship. Unless otherwise expressly authorized in writing, Products are supplied for research use only. No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than the original purchaser of the Product ("Buyer").

No other warranties, express or implied, are granted, including without limitation, implied warranties of merchantability, fitness for any particular purpose, or non infringement. Buyer's exclusive remedy for non-conforming Products during the warranty period is limited to replacement of or refund for the non-conforming Product(s).

There is no obligation to replace Products as the result of (i) accident, disaster or event of force majeure, (ii) misuse, fault or negligence of or by Buyer, (iii) use of the Products in a manner for which they were not designed, or (iv) improper storage and handling of the Products.

Current product instructions are available at *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.

Pierce Biotechnology 3747 N. Meridian Road | PO Box 117 Rockford, IL 61105 USA | (815) 968-0747 (815) 968-7316 fax | www.thermoscientific.com/pierce