INSTRUCTIONS

Reacti-Bind™ NeutrAvidin™ Coated 96-Well Plates

PIERCE
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0611.2

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

Introduction

The Reacti-Bind™ 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 (25 mM Tris, 150 mM NaCl; pH 7.2; Product No. 28376), 0.1% BSA, 0.05% Tween®-20; alternatively, use Blocker™ BSA (Product No. 37520) supplemented with 0.05% Tween®-20
- Biotinylated capture antibody adjusted to 10 µg/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 200 µl of Wash Buffer. Add 100 µl of the biotinylated capture antibody to each well and incubate for 2 hours 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 at room temperature.
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. Incubate plate for 30 minutes at room temperature.
5. Wash each well three times with 200 µl 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 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 200 µl of wash buffer (e.g., TBS).
2. Prepare a 1 mg/ml solution of the biotinylated enzyme. Make 1:2 serial dilutions using a 1:1,000 dilution for the first well. Incubate the wells for 1 hour at room temperature.
3. Wash each well three times with 200 µl of TBS containing 0.05%Tween®-20.
4. Incubate with 100 µl 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 405 nm.

Related Pierce Products

37070	**SuperSignal® ELISA Pico Chemiluminescent Substrate,** 100 ml, peroxidase substrate
15169	**QuantaBlu™ Fluorogenic Peroxidase Substrate Kit**
34028	**1-Step™ Ultra TMB-ELISA,** 250 ml, colorimetric peroxidase substrate
37621	**1-Step™ PNPP,** 100 ml, colorimetric phosphatase substrate
29339	**ImmunoPure® Biotinylated Alkaline Phosphatase,** 1 mg
29139	**ImmunoPure® Biotinylated Horseradish Peroxidase,** 5 mg
15075	**ImmunoWare™ Reagent Reservoirs,** 200/pkg.
15082	**ImmunoWare™ Microtube Racked System,** 960 tubes
15036	**Sealing Tape for 96-Well Plates,** 100/pkg.

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.