

Reacti-Bind™ Biotin Binding 96-Well Plate Sample Pack

15115

0733.1

Number	Description
15115	Reacti-Bind Biotin Binding Plate Sample Pack , contains one of each of the following clear, 8-well strip plate: Streptavidin Coated Plate blocked with SuperBlock® Blocking Buffer (Reorder #15120) Activation Level: 100 µl Binding Capacity: 5 pmol D-biotin/well NeutrAvidin™ Coated Plate blocked with SuperBlock® Blocking Buffer (Reorder #15127) Activation Level: 100 µl Binding Capacity: 15 pmol D-biotin/well Streptavidin Coated Plate blocked with Blocker™ BSA (Reorder #15121) Activation Level: 200 µl Binding Capacity: 10 pmol D-biotin/well NeutrAvidin™ Coated Plate blocked with Blocker™ BSA (Reorder #15128) Activation Level: 200 µl Binding Capacity: > 15 pmol D-biotin/well

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

Thermo Scientific Reacti-Bind Biotin Binding Plate Sample Pack is ideal for developing assays. This sample pack contains streptavidin and NeutrAvidin™ Protein coated on microplates that are preblocked with either SuperBlock® Blocking Buffer or Blocker™ BSA. This variety allows researchers to determine which plate produces the best signal-to-noise ratio for a specific experimental system. Once optimal conditions are empirically determined, the plate that provides the best results can be purchased in bulk for the remainder of the study.

Example ELISA Protocol

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 1-Step™ Ultra TMB-ELISA (Product No. 34028) 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.
2. Add 100 µl of the biotinylated capture antibody to each well and incubate for 2 hours with shaking at room temperature.
3. Wash each well three times with 200 µl of Wash Buffer.
4. Make a serial dilution of the antigen and add 100 µl to each well. Incubate plate for 30 minutes with shaking at room temperature.

5. Wash each well three times with 200 µl of Wash Buffer.
6. Add 100 µl of the primary antibody to each well and incubate plate for 30 minutes at room temperature.
7. Wash each well three times with 200 µl of Wash Buffer.
8. Add 100 µl of the enzyme-labeled secondary antibody to each well. Incubate plate for 30 minutes with shaking at room temperature.
9. Wash each well three times with 200 µl of Wash Buffer.
10. Follow the manufacturer's instructions for the specific detection system.

Procedure for Determining Binding Activity of the Coated Plates

The binding activity of the plates can 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 Thermo Scientific Products

15120	Reacti-Bind™ Streptavidin Coated Strip Plate , with SuperBlock® Blocking Buffer, 5 plates
15121	Reacti-Bind™ Streptavidin Coated Strip Plate , with Blocker™ BSA, 5 plates
15127	Reacti-Bind™ NeutrAvidin™ Coated Strip Plate , with SuperBlock® Blocking Buffer, 5 plates
15128	Reacti-Bind™ NeutrAvidin™ Coated Strip Plate , with Blocker™ BSA, 5 plates
34028	1-Step™ Ultra TMB-ELISA , 250 ml, colorimetric peroxidase substrate
37621	1-Step™ PNPP , 100 ml, colorimetric phosphatase substrate
29339	Biotinylated Alkaline Phosphatase , 1 mg
29139	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

- Chalet, I and Wolf, F.J. (1974). The properties of streptavidin, a biotin-binding protein produced by *Streptomyces*. *Arch. Biochem. Biophys.* **106**:1-5.
- Ferre-Aubineau, V., *et al.* (1995). Colorimetric microwell plate hybridization assay using monoclonal antibody for detection of an amplified human immunodeficiency virus target. *J. Virol. Methods.* **55**:145-51.
- 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.
- Jalanko, A., *et al.* (1991). Screening for defined cystic fibrosis mutations by solid phase mini-sequencing. *Clin. Chem.* **38**:39-43.
- Koskiniemi, M., *et al.* (1997). Luminometric microplate hybridization for detection of varicella-zoster virus PCR product from cerebrospinal fluid. *J. Virol. Methods.* **63**:71-9.

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

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