Pierce® Streptavidin Coated 96-Well Plates

INSTRUCTIONS

Introduction
The Thermo Scientific Pierce Streptavidin Coated Plates are made of polystyrene and are ideal for binding assays using biotinylated molecules. 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 streptavidin coated plates are available in clear for colorimetric assays, white for chemiluminescent assays, and black for fluorescent assays.

Example ELISA Procedure
The following protocol describes a generalized enzyme-linked immunosorbent assay using a biotinylated capture antibody. Please see the reference list for other possible applications using streptavidin-coated microplates.

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
- Biotinylated capture antibody adjusted to 10μg/mL, or other appropriate concentration, with Wash Buffer
- Antigen adjusted to appropriate concentration with Wash Buffer

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.

Number Description
15120 Pierce Streptavidin Coated Plate (clear, 8-well strips), 5 plates
15122 Pierce Streptavidin Coated Plate (clear, 8-well strips), 5 × 5 plates
15124 Pierce Streptavidin Coated Plate (clear, 96-well), 5 plates
15126 Pierce Streptavidin Coated Plate (clear, 96-well), 5 × 5 plates
15118 Pierce Streptavidin Coated Plate (white, 96-well), 5 plates
15119 Pierce Streptavidin Coated Plate (black, 96-well), 5 plates
15121 Blocking Buffer: These plates are supplied blocked with SuperBlock® Blocking Buffer
   Binding Capacity: ~5pmol D-biotin/well
   Activation Level: 100µL
15125 Pierce Streptavidin Coated Plate (clear, 8-well strips), 5 plates
15218 Pierce Streptavidin Coated Plate (white, 96-well), 5 plates
15219 Blocking Buffer: These plates are supplied blocked with Blocker™ BSA
   Binding Capacity: ~10pmol D-biotin/well
   Activation Level: 200µL

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

The binding activity of the plates can 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.
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

<table>
<thead>
<tr>
<th>Product Code</th>
<th>Product Name</th>
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</thead>
<tbody>
<tr>
<td>37070</td>
<td>SuperSignal® ELISA Pico Chemiluminescent Substrate, 100mL</td>
</tr>
<tr>
<td>15169</td>
<td>QuantaBlu™ Fluorogenic Peroxidase Substrate Kit</td>
</tr>
<tr>
<td>34028</td>
<td>1-Step™ Ultra TMB-ELISA, 250mL</td>
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<tr>
<td>37621</td>
<td>1-Step PNPP, 100mL</td>
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<tr>
<td>29339</td>
<td>Biotinylated Alkaline Phosphatase, 1mg</td>
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<tr>
<td>29139</td>
<td>Biotinylated Horseradish Peroxidase, 5mg</td>
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<tr>
<td>15075</td>
<td>Reagent Reservoirs, 200/pkg</td>
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<tr>
<td>15082</td>
<td>Microtube Racked System, 960 tubes</td>
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<tr>
<td>15036</td>
<td>Sealing Tape for 96-Well Plates, 100/pkg</td>
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<tr>
<td>45360</td>
<td>Pierce Streptavidin Coated Plate Immunoprecipitation Kit</td>
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<tr>
<td>21425</td>
<td>EZ-Link® Sulfo-NHS-Biotinylation Kit</td>
</tr>
<tr>
<td>21335</td>
<td>EZ-Link Sulfo-NHS-LC-Biotin, 100mg</td>
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General References

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

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