INSTRUCTIONS

**ImmunoPure® Protein G,**
Alkaline Phosphatase Conjugated

**31399**

<table>
<thead>
<tr>
<th>Number</th>
<th>Description</th>
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<tr>
<td>31399</td>
<td><strong>ImmunoPure® Protein G, Alkaline Phosphatase Conjugated</strong>, 0.5 mg</td>
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Source: Recombinant Protein G and lyophilized calf intestinal phosphatase

Storage: Upon receipt store frozen at -20°C. Product is shipped with dry ice.

**Introduction**

Protein G is a bacterial cell wall protein isolated from group G *Streptococci*. Protein G binds to many mammalian immunoglobulins mostly through their Fc regions. Native protein G has two immunoglobulins binding sites as well as sites for albumin and cell surface binding; however, the albumin and cell surface binding sites have been eliminated from recombinant protein G (rProtein G, 22.6 kDa), which minimizes nonspecific binding. Typically, binding to Protein G is achieved using 0.1 M sodium acetate buffer at pH 5.4; however, the optimal pH for binding is species dependent.

Alkaline phosphatases are a widely distributed family of isozymes present in many species and tissues. This enzyme has an optimal pH of 9.0-9.6, is activated by divalent cations, and inhibited by cysteine, cyanides, arsenate, and divalent cation chelators. Alkaline phosphatase is a good choice when high endogenous peroxidase levels preclude the use of horseradish peroxidase, a condition often encountered in cryostat sections.

**Note:** The following protocols are general examples of applications. Each specific application and experimental system must be empirically optimized.

**Example Western Blotting Procedure**

For each step, use sufficient reagent to cover the entire membrane. Agitate the membrane when performing the procedure.

**Materials**

- Tris-buffered saline (TBS): 25 mM Tris, 150 mM NaCl; pH 7.2 (Product No. 28376)
- Blocking Buffer: TBS containing 1% BSA (Blocker™ BSA in TBS, Product No. 37520)
- Wash Buffer: TBS containing 0.1% BSA
- Primary antibody adjusted to appropriate concentration with Blocking Buffer
- ImmunoPure® Protein G, Alkaline Phosphatase Conjugated diluted to 0.5 µg/ml with wash buffer
- Enzyme substrate: for example, 1-Step™ NBT/BCIP (Product No. 34042)

**Method**

1. Place membrane containing transferred proteins in a flat-bottom dish. Add Blocking Buffer to the membrane and incubate for one hour.
2. Rinse membrane three times with Wash Buffer. Add the primary antibody to the membrane and incubate for one hour at room temperature.
3. Rinse membrane three times with Wash Buffer. Add the appropriate concentration of alkaline phosphatase conjugated Protein G to the membrane and incubate for one hour.
4. Rinse membrane three times with Wash Buffer. Add the detection substrate and develop according to the manufacturer’s instructions.
Example ELISA Procedure

Materials

- Coating Buffer: 0.2 M sodium bicarbonate, pH 9.4 (Product No. 28382)
- Tris-buffered saline (TBS): 25 mM Tris, 150 mM NaCl; pH 7.2 (Product No. 28376)
- Wash Buffer: TBS containing 0.05% Tween®-20
- Blocking Buffer: TBS containing 0.05% Tween®-20 and 1% BSA (Blocker™ BSA in TBS, Product No. 37520)
- Primary antibody adjusted to appropriate concentration with Blocking Buffer
- ImmunoPure® Protein G, Alkaline Phosphatase Conjugated diluted to 0.5 µg/ml with Wash Buffer

Note: Optimal binding to Protein G occurs at pH 5.0, but physiological buffers are also suitable for many antibodies
- Enzyme Substrate: for example, use the Phosphatase Substrate Kit (Product No. 37620)

Method

1. Prepare an antigen solution at approximately 10 µg/ml in Coating Buffer. Add 150 µl of antigen solution to each microplate well. Incubate for 1 hour at 37°C or 18 hours at 4°C.
2. Wash plate three times with 200 µl of Wash Buffer.
3. Add 200 µl of Blocking Buffer and incubate for 1 hour at 37°C. Wash plate three times with 200 µl of Wash Buffer.
4. Add 150 µl of the primary antibody to each well and incubate for 2 hours at room temperature.
5. Wash three times with 200 µl of Wash Buffer. Add 150 µl of 0.1 µg/ml of alkaline phosphatase conjugated Protein G to the wells and incubate for 2 hours at room temperature.
6. Wash plate four times with 200 µl of Wash Buffer. Add the detection substrate and develop according to the manufacturer’s instructions.

Related Pierce Products

<table>
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<tr>
<th>Code</th>
<th>Description</th>
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<tbody>
<tr>
<td>28320</td>
<td>Surfact-Amps® 20 Purified Detergent Solution, 6 x 10 ml, contains 10% Tween®-20</td>
</tr>
<tr>
<td>37621</td>
<td>1-Step™ PNPP, 100 ml, colorimetric phosphatase substrate</td>
</tr>
<tr>
<td>15075</td>
<td>ImmunoWare™ Reagent Reservoirs, 200/pkg.</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>21059</td>
<td>Restore™ Western Blot Stripping Buffer, 500 ml</td>
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<tr>
<td>21065</td>
<td>Erase-It® Background Eliminator Kit, for eliminating background from X-ray film</td>
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<tr>
<td>88018</td>
<td>Nitrocellulose Membrane, 0.45 µm, 33 cm x 3 m, 1 roll</td>
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<tr>
<td>88600</td>
<td>Western Blotting Filter Paper, 8 cm x 10.5 cm, 100 sheets</td>
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<tr>
<td>24580</td>
<td>MemCode™ Reversible Protein Stain Kit for Nitrocellulose Membranes</td>
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<tr>
<td>24585</td>
<td>MemCode™ Reversible Protein Stain Kit for PVDF Membranes</td>
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References


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