Introduction

Protein L is an immunoglobulin-binding protein that was isolated from the bacteria *Peptostreptococcus magnus* and is now produced by recombinant methods. Protein L binds to immunoglobulin kappa light chains without interfering with the antigen-binding site and binds a wider range of Ig classes and subclasses than other antibody-binding proteins, such as Protein A or Protein G. Protein L binds to all classes of Ig (i.e., IgG, IgM, IgA, IgE and IgD).

Important Product Information

- Protein L only binds to immunoglobulins containing light chains of type kappa I, III, and IV in human and kappa I in mouse. Protein L also may be specific for certain kappa subgroups in other species. Protein L also binds single chain variable fragments (scFv) and Fab fragments.
- Protein L binds weakly to rabbit immunoglobulins and does not bind immunoglobulins from bovine, goat or sheep; nor does it bind to lambda light chains.
- The following protocols are example applications for biotinylated Protein L. For best results, develop an optimized procedure for specific applications.
- Reconstitute Pierce Recombinant Biotinylated Protein L with ultrapure water and store in single-use aliquots at -20°C.

Example Western Blotting Protocol

Materials Required

- Phosphate-buffered saline (PBS, Product No. 28372)
- Blocking Buffer: 1% bovine serum albumin (BSA) in PBS (Thermo Scientific Blocker BSA in PBS, Product No. 37525)
- Wash Buffer: PBS containing 0.3% BSA
- Primary antibody diluted to the appropriate concentration
- Biotinylated Protein L diluted to 0.5µg/mL in PBS
- Enzyme conjugate: Labeled Thermo Scientific NeutrAvidin Protein or Streptavidin diluted to 2µg/mL with Blocking Buffer
- Appropriate enzyme substrate
Procedure
Note: Perform incubation steps at room temperature. During each step, use sufficient reagent to cover the entire membrane. Agitate the membrane when performing the procedure.

1. Place membrane containing transferred protein in a flat-bottom dish. Add Blocking Buffer and incubate for 1 hour.
2. Rinse membrane three times for 10 minutes with Wash Buffer. Add the primary antibody and incubate for 1 hour.
3. Rinse membrane three times for 10 minutes with Wash Buffer. Add the diluted Biotinylated Protein L and incubate for 1 hour.
4. Rinse membrane three times for 10 minutes with Wash Buffer. Add the enzyme conjugate and incubate for one hour.
5. Rinse membrane three times for 10 minutes with Wash Buffer. Add the substrate and develop according to the manufacturer’s instructions.

Example ELISA Protocol
Materials Required
• Coating Buffer: 0.2M carbonate-bicarbonate, pH 9.4 (Product No. 28382)
• Phosphate-buffered saline (PBS, Product No. 28372)
• Blocking Buffer: 1% bovine serum albumin (BSA) in PBS (Blocker™ BSA in PBS, Product No. 37525) containing 0.05% Tween™-20 Detergent
• Wash Buffer: PBS containing 0.05% Tween-20 Detergent
• Antigen (150µL of 1-10µg/mL in Coating Buffer) for coating onto the plate
• Primary antibody diluted to appropriate concentration
• Biotinylated Protein L diluted to 1µg/mL with PBS
• Enzyme conjugate: Labeled NeutrAvidin™ Protein or Streptavidin diluted to 0.1µg/mL with PBS
• Appropriate enzyme substrate

Procedure
1. Coat antigen onto each microplate well. Incubate for 1 hour at 37°C or 18 hours at 4°C.
2. Rinse each well three times with 150µL of Wash Buffer. Add 150µL Blocking Buffer to each well and incubate for 30-60 minutes at 37°C.
3. Discard Blocking Buffer and rinse each well three times with 150µL of Wash Buffer. Add 150µL of the primary antibody and incubate for 1 hour at 37°C.
4. Rinse each well three times with 150µL of Wash Buffer. Add 150µL Biotinylated Protein L to each well and incubate for 1 hour at 37°C.
5. Rinse each well three times with 150µL of Wash Buffer. Add 150µL of the enzyme conjugate to each well and incubate for 1-2 hours at 37°C.
6. Rinse each well three times with 150µL of Wash Buffer. Add the substrate and develop according to the manufacturer’s instructions.

Additional Information
Please visit the website for additional information relating to this product including the following items:
• Tech Tip #34: Binding characteristics of Protein A, Protein G, Protein A/G and Protein L
• Tech Tip #43: Protein stability and storage
General References


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