

ImmunoPure[®] Protein L- Peroxidase Conjugated

32420

0780w

Product Description

Number

32420

Description

ImmunoPure[®] Protein L-Peroxidase Conjugated, 0.5 mg

This product is provided lyophilized.

After reconstitution, aliquot and store at -20°C.

Introduction

Protein L is an immunoglobulin-binding protein that originally comes from the bacteria *Peptostreptococcus magnus*, but is now produced recombinantly. Protein L has the unique ability to bind through kappa light chain interactions without interfering with an antibody's antigen-binding site. This gives Protein L the ability to bind a wider range of Ig classes and subclasses than other antibody-binding proteins such as Protein A or Protein G. Protein L will bind to all classes of Ig (IgG, IgM, IgA, IgE and IgD). This also gives Protein L the unique ability to bind Single Chain Variable Fragments (ScFv) and Fab fragments.

Antibody Binding Information for Protein L

- Binds to all classes of Ig (IgG, IgM, IgA, IgE and IgD)
- Binds to the V_L region of kappa light chains without interfering with antigen binding
- Does not bind lambda light chains, not even weakly
- Binds only certain kappa light chains in human and mouse. It binds kappa I, III, and IV in human and kappa I in mouse
- It is possible that Protein L is also specific for only certain kappa subgroups in other species. Pierce currently only has binding information on human and mouse kappa chains
- Binds ScFv
- Binds weakly to rabbit Igs
- Does not bind bovine, goat or sheep Igs

ImmunoPure[®] Recomb[®] Protein L is a 35,000 molecular weight, recombinant form of Protein L. ImmunoPure[®] Horseradish Peroxidase has been covalently coupled to ImmunoPure[®] Recomb[®] Protein L to produce ImmunoPure[®] Protein L-Peroxidase Conjugated. ImmunoPure[®] Recomb[®] Protein L-Peroxidase Conjugated can be used in Western blotting or ELISA procedures.

Example Protocols for ELISA and Immunoblotting

Peroxidase-labeled Protein L can be substituted for labeled secondary antibody in ELISA, immunoblotting and immunocytochemistry procedures requiring detection of immunoglobulins or their fragments. Described below are general protocols for these procedures. A more extensive protocol should be developed for your particular application.

Example Protocol I - Blotting

Materials

- A. Phosphate Buffered Saline, pH 7.2 (PBS) (Prod. No. 28372)
- B. Blocking Buffer: e.g., PBS containing 1% bovine serum albumin (Prod. No. 37525)
- C. Wash Buffer: PBS containing 0.1% BSA.
- D. Primary antibody: Anti-antigen antibody (adjust to appropriate concentration with Wash Buffer)
- E. ImmunoPure[®] Protein L-Peroxidase Conjugated: Dilute to approximately 0.5 µg/ml with Wash Buffer.
- F. Enzyme Substrate: Follow specific substrate's directions for preparation.

Methods

1. After Western transfer of the protein(s) to a nitrocellulose membrane, place the membrane in a flat-bottom dish.
2. Incubate the membrane for one hour in Blocking Buffer.
3. Rinse the membrane 3 x 10 minutes with Wash Buffer.
4. Incubate the membrane for one hour with primary antibody.
5. Rinse the membrane 3 x 10 minutes with Wash Buffer.
6. Incubate the membrane for one hour with ImmunoPure[®] Recomb[®] Protein L- Peroxidase Conjugated.
7. Rinse the membrane 3 x 10 minutes with Wash Buffer.
8. Add an appropriate substrate for HRP and allow to react until sufficient signal develops. (Follow directions for given substrate.)

Example Protocol II - ELISA

This example ELISA procedure involves coating microwell plates with the antigen, incubating with the primary antibody, incubating with an enzyme-labeled secondary antibody and finally adding the appropriate substrate. A colored reaction product is used to quantitate the amount of antigen in a sample.

Materials

- A. Coating Buffer: 0.2 M sodium carbonate-bicarbonate, pH 9.4 (Prod. No. 28382)
- B. Phosphate Buffered Saline, pH 7.2 (Prod. No. 28372)
- C. PBS/Tween[®]-20 detergent: PBS containing 0.05% Tween[®]-20 detergent.
- D. BSA Wash Buffer: PBS/0.05% Tween[®]-20, 0.1% BSA, pH 7.4.
- E. BSA Blocking Buffer: PBS/0.05% Tween[®]-20, 1% BSA.
- F. Primary antibody: Adjust to appropriate concentration with Wash Buffer.
- G. ImmunoPure[®] Protein L-Peroxidase Conjugated (dilute to 0.5 µg/ml with Wash Buffer).
- H. Substrate for HRP: Follow substrate's directions for preparation.

Method

1. Prepare antigen solution at approximately 10 µg/ml in Coating Buffer.
2. Place 150 µl of antigen in Coating Buffer in each well of the microwell plate.
3. Incubate 1 hour at 37°C (or 18 hours at 4°C).

4. Rinse 3 x 150 μ l with PBS/Tween[®]-20.
5. Incubate for 1 hour at 37°C with BSA Blocking Buffer to block the remaining protein binding sites on the microwell plate.
6. Add 150 μ l of a 1 μ g/ml solution of primary antibody in Wash Buffer to each well.
Note: Optimum concentration must be determined for each antibody.
7. Incubate for 2 hours at room temperature (RT).
8. Rinse 3 x 150 μ l with PBS/Tween[®]-20 to remove unbound antibody.
9. Apply 150 μ l of 0.1 μ g/ml solution of ImmunoPure[®] Protein L-Peroxidase Conjugated.
10. Incubate for 2 hours at RT.
11. Rinse each well with 4 x 150 μ l of PBS/Tween[®]-20.
12. Add HRP substrate. (Follow directions for given substrate.)

References

1. Björck, L. (1988). Protein L - a novel bacterial cell wall protein with affinity for immunoglobulin light chains. *J. Immunol.* **140**, 1194-1197.
2. Kastern, W., Sjöbring, U. and Björck, L. (1992). Structure of *peptostreptococcal* protein L and identification of a repeated immunoglobulin light chain-binding domain. *J. Biol. Chem.* **267**, 12820-12825.
3. Nilson, B.H.K., Lögdberg, L., Kastern, W., Björck, L. and Åkerström, B. (1993). Purification of antibodies using protein L-binding framework structures in the light chain variable domain. *J. Immunol. Meth.* **164**, 33-40.
4. Åkerström, B. and Björck, L. (1989). Protein L - an immunoglobulin light chain-binding bacterial protein. Characterization of binding and physicochemical properties. *J. Biol. Chem.* **264**, 19740-19746.

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Affinity Purification of Polyclonals/Monoclonals

From Ascites or Cell Culture and Polyclonal IgG Antibodies

	Protein A	Ig Binding Protein Protein G	Protein L
Human IgG	s	s	s
Mouse IgG	s	s	s
Rabbit IgG	s	s	w
Chicken IgY	nb	nb	nb
Rat IgG	w	m	s
Goat IgG	w	s	nb
Sheep IgG	w	s	nb
Bovine IgG	w	s	nb
Porcine IgG	s	w	s

Antibody Subclasses and Non-IgGs

	Protein A	Protein G	Protein L
Human Fab	w	w	s
Human ScFv	w	nb	s
Human IgM	w	nb	s
Human IgE	m	nb	s
Human IgD	nb	nb	s
Human IgA	w	nb	s
Human IgG1	s	s	s
Human IgG2	s	s	s
Human IgG3	w	s	s
Human IgG4	s	s	s
Mouse IgM	nb	nb	s
Mouse IgG1	w	m	s
Mouse IgG2a	s	s	s
Mouse IgG2b	s	s	s
Mouse IgG3	s	s	s
Bovine IgG1	w	s	nb
Bovine IgG2	s	s	nb
Rat IgG1	w	m	s
Rat IgG2a	nb	s	s
Rat IgG2b	nb	w	s
Rat IgG2c	s	s	s
Sheep IgG1	w	s	nb
Sheep IgG2	s	s	nb
Goat IgG1	w	s	nb
Goat IgG2	s	s	nb

w = weak binding, m = medium binding, s = strong binding, nb = no binding, ? = information not available