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I. DESCRIPTION

Protein L is an immunoglobulin-binding protein that binds to immunoglobulin κ 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). Protein L also binds single chain variable fragments (Scfv) and Fab fragments.

Protein L Resin was made by covalently coupling purified protein L to agarose support. This leak-resistant coupling method ensures excellent gel stability and binding characteristics.

II. KEY FEATURES

- ◆ Broad Ig binding spectrum
- ◆ High capacity of more than 15 mg immunoglobulin per ml of resin
- ◆ Cross-linked agarose media

III. PROPERTIES OF PROTEIN L RESIN

Ligand	Purified protein L
Number of Ig binding sites per ligand	5
Mr of ligand	Approximately 42
PI of ligand	4.57
Degree of substitution	Approximately 2 mg protein L/ml
Static binding capacity	>15 mg porcine Ig/ml drained medium
Stability	37°C, 7days
Matrix spherical	Agarose, 4% cross-linked
Average particle size	90 μm (45-165 μm)
Sanitization	Washing the packed column with 70% ethanol
Storage	20% ethanol at 4 to 8°C

IV. IMMUNOGLOBULIN PURIFICATION PROCEDURE

Before use, prepare the following two solutions:

1. Binding buffer A:
 - Na₂HPO₄ 20 mM
 - NaCl 0.15 M, adjust pH to 7.0.
2. Elution buffer B:
 - Citric acid 0.1 M, adjust pH to 3.0.



This procedure is for a column of 0.5 ml bed volume. The volumes of reagents can be scaled up or down according to the size of the column.

1. Mix the slurry by gently inverting the bottle several times to suspend the resin completely.
2. Use a pipette to transfer appropriate volume of Protein L Resin slurry to a column. Allow the resin to settle and the storage buffer to drain from the column.
3. Add 5 ml of binding buffer A to equilibrate the Protein L Resin.
4. Dilute the sample with the same volume or more of binding buffer A before applying onto the protein L column to maintain optimal ionic strength for binding.
5. Wash the column with 10 ml of binding buffer A.
6. Elute the antibody with 10 ml of elution buffer B. Immediately neutralize the eluted fractions with 1 M Tris-HCl, pH 8.5 to pH 7.4.

Regeneration of the column.

7. Regenerate column by washing the column with 10 ml of elution buffer B followed by equilibration of the column with 5 ml of binding buffer A. Columns can be regenerated up to 10 times without significant loss of binding capacity.
8. For storage, wash column with 5 ml of PBS containing 0.02% sodium azide. Store column upright at 4°C.

V. APPLICATION EXAMPLE

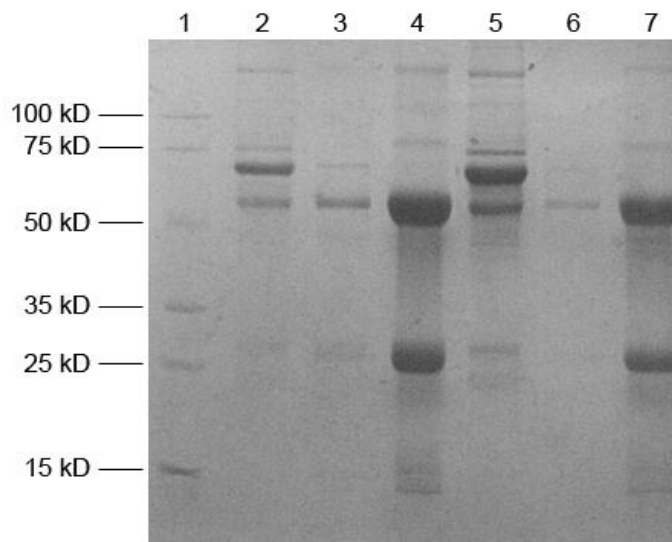


Fig.1. SDS-PAGE analysis of antibody purification using GenScript Protein L Resin (L00239)

1. Smart Protein Marker (GenScript, MM0900)
2. Flow (GenScript)
3. Wash (GenScript)
4. Elute (GenScript)
5. Flow (X corporation)
6. Wash (X corporation)
7. Elute (X corporation)



VI. TROUBLESHOOTING

Problem	Possible Cause	Solution
Flow of the column is exceedingly slow (i.e., <0.5 ml/minute)	Outgassing of buffers or sample on the column, which results in blockage of gel pores with microscopic air bubbles.	Degas buffers and remove air bubbles from column.
Considerable antibody purified, but no specific antibody of interest detected	Antibody of interest is at very low Concentration.	Use serum-free medium for cell supernatant samples.
		Affinity purify the antibody using the specific antigen coupled to an affinity support.
Antibody of interest purified, but it is degraded (as determined by lack of function in downstream assay)	Antibody is sensitive to low-pH elution buffer.	Increase pH of elution buffer.
	Downstream application is sensitive to neutralized elution buffer.	Desalt or dialyze eluted sample into suitable buffer.
No antibody detected in any elution fraction	Sample devoid of antibody species or subclass that binds to protein L.	Refer to the binding characteristics table for protein L.

VII. ORDER INFORMATION

Protein L Resin 2.5 ml (5 ml of 50% slurry): L00239

For Research Use Only.

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