Protein L Superflow 4

Sterogene's Protein L Superflow 4 is a versatile affinity resin designed for easy and single step purification of all the classes of antibodies (IgA, IgD, IgE, IgG and IgM) and antibody fragments (e.g., Fabs and single chain variable fragments). It strongly binds with IgGs from human, pig, mouse and rat. Protein L Superflow 4 is made by covalently immobilizing recombinant Protein L containing 5 IgG binding domains to highly crosslinked 4% agarose beads through epoxy chemistry. The Protein L coupled beads are stable at pH 2-13 and show no leaching in commonly used buffers and different pH conditions. Protein L Superflow 4 can be extremely helpful in purification of monoclonal antibodies (with kappa light chains) from cell cultures, where bovine serum is used as media supplements because Protein L doesn't bind with bovine immunoglobulins.

Protein L is a bacterial surface expressed protein isolated from *Peptostreptococcus magnus* and just like Protein A and Protein G it binds to immunoglobulins but unlike Protein A and G, Protein L binds to immunoglobulins via the light chain. Protein L binds to kappa light chain antibodies without interfering with the antigen binding site. Protein L contains five homologous Ig or kappa binding domains. Protein L efficiently interacts with all classes of antibodies (IgG, IgM, IgA, IgD and IgE) but can't be used as a universal Ab purification ligand because its interactions are limited to only kappa light chain containing Abs and doesn't bind to Abs with lambda light chains.

Protein L Superflow 4 resin is supplied as 50% slurry (in 20% ethanol).

Upon receiving, store the resin at 2-8 °C. Do not frreze.

Applications: Purification of monoclonal, polyclonal antibodies, Fabs, single chain antibodies. Immunoprecipitation.

Key features:

Matrix support: 4% highly crosslinked agarose

Immobilized ligand: recombinant Protein L

Ligand density: 5 mg/ml of resin

Binding capacity: 20 mg of IgG/ml of settled resin

Storage buffer: PBS with 20% ethanol

Elution buffer: 0.1 M glycine pH 3.

Protocol:

Materials required:

Sterogene Protein L Superflow 4 resin

Disposable columns

Equilibration buffer: 10 mM phosphate buffer with 150 mM NaCl (pH 7.0)

Wash buffer A: 10 mM phosphate with 150 mM NaCl (pH 7.5)

Wash buffer B: 10 mM phosphate buffer with 500 mM NaCl (pH 7.5)

Elution buffer: 0.1 M glycine, pH 3

Neutralization buffer: 1.0 M PBS or 1.0 M Tris (pH 8.5)

Remove the Protein L Superflow 4 resin from 4 °C and equilibrate it at room temperature along with the required buffers. Below is the protocol for purification of IgG from serum using 1 ml of Protein L Superflow 4 in a column.

1. Pack 2 ml of Protein L Superflow 4 slurry in appropriate disposable column and let the column drain completely.
2. Wash the beads with 10 ml of equilibration buffer at high flow rate, let the column drain properly.
3. Dilute the loading sample (1:1) in equilibration buffer and apply to the column at slow flow rate. Check the loading sample for any precipitation after dilution. If required, centrifuge the sample (15000 rpm at room temperature for 15 min) to remove any precipitate and load the clear supernatant to the affinity column.
4. Wash the column with 10 ml of wash buffer A followed by 10 ml of wash buffer B. Monitor the wash fractions at OD_{280} for presence of any protein. Additional wash steps can be performed until the baseline is reached.
5. Elute the bound IgGs with 5 ml of 0.1 M glycine (pH 3.0), elution should be done at slow flow rate and in small fractions (500 µl).
6. Immediately neutralize the acidic pH of the eluted samples to physiological pH by adding 50 µl of neutralization buffer to each fraction and check their OD_{280} to calculate the IgG concentration.
7. Run all the fractions on SDS-PAGE to check the purity/homogeneity of the eluted protein, pool all the pure fractions and dialyze in the required buffer.
8. The Protein L Superflow 4 column can be regenerated by washing with 20 ml of wash buffer B and can be further used multiple times (>10 times) without loss of any significant activity.
9. For storage, wash the beads with 5 ml of deionized water containing 0.2% sodium azide and store the column at 4 °C with 2-3 ml of same 0.02% sodium azide solution.