Protein G Superflow 4

Sterogene's Protein G Superflow 4 is a high-quality affinity chromatographic resin used for one-step purification of various classes/subclasses of immunoglobulins at small to large from biological samples and over-expressed media. Our stable and simple to use Protein G Superflow 4 resin is made by covalently immobilizing purified recombinant Protein G to 4% highly crosslinked agarose beads through epoxy chemistry. The resin shows no leaching of Protein G in commonly used buffers and in a pH range from 2-13. Sterogene's Protein G Superflow 4 has a high static binding capacity of > 35 mg of IgG per mL of settled resin and can be used several times without significant loss in yield.

Protein G is a cell-wall protein expressed in certain *Streptococcus* bacteria belonging to Group G and C classes. Protein G from group G *Streptococcus* has 3 and group C *Streptococcus* has 2 IgG binding domains. In terms of binding, Protein G shows broader range and higher affinity for several IgGs than Protein A. It can be used for the purification of certain human monoclonal or polyclonal antibodies which are not recognized by Protein A. Protein G binds strongly to IgG through its Fc region and shows weak binding to the Fab region, but it fails to recognize human IgM, IgD and IgA. Protein G shows optimal binding with IgG at low pH (pH 5.0-5.5) but near physiological pH (~ pH 7.0) can be used for purification purposes.

Protein G Superflow 4 resin is designed for versatile usage. It can be used in small to large scale IgG purification using FPLC or gravity flow columns and can also be used efficiently for immunoprecipitation reactions.

Protein G Superflow 4 is supplied as 50% slurry in deionized water containing 0.02% sodium azide.

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

Key features:

Matrix support: 4% crosslinked agarose beads

Immobilized ligand: recombinant Protein G (3 IgG binding domains)

Ligand density: 5 mg Protein G/mL of agarose beads

Static Binding capacity: 35 mg of human IgG/mL of resin

Storage buffer: 0.02% sodium azide solution in deionized water

Elution buffer: 0.1 M glycine, pH 2-3.

Protocol for purification of IgG:

Materials required:

Sterogene Protein G 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 8.0)

Remove the Protein G 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 G Superflow 4 in a column.

1. Pack 2 mL of Protein G 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 G 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.