Protein A Ultraflow is manufactured by covalently immobilizing recombinant Protein A to a 4% agarose Ultraflow resin through a stable linkage. The static binding capacity is 40mg human IgG per ml resin. Protein A Ultraflow is made in the particle size range of 40-160µm and is recommended for clarified feedstreams. The resin is stable with commonly used buffers and reagents including 1M Na_2CO_3, urea, high salt, guanidine, 50% ethanol, and 50% methanol. The pH stability range is pH 2-10.

IgG antibodies exhibit a species and subclass specific affinity for Protein A. Antibody binding to Protein A Ultraflow is independent of culture medium composition; it is affected only by Fc domain heterogeneity. Therefore, the IgG can be directly applied to the column after clarification of the sample (e.g. filtration); special binding buffers are not required for optimal antibody binding to immobilized protein A. This is different from ion exchange media where the ionic strength and pH of the harvest need to be changed before column loading.

Choice of Buffer

The resin is shipped in 20% ethanol storage buffer. The recommended equilibration buffer for Protein A Ultraflow is phosphate-buffered saline, pH 7.0-7.2, (PBS).

Optimal Elution

IgG can be eluted from Protein A Ultraflow with any of the standard acidic elution media (pH 2.2-3.5). If low pH elution is not an option due to the stability of the antibody, then we recommend using a gentle antibody elution buffer such as ActiSep Elution Medium (Sterogene #9701).

Column Preparation

Prime the column with the selected starting buffer. Equilibrate by passing 5 bed volumes of PBS through the column at a rate of 300 cm/hr.

Sample Loading

The recommended flow rate range for sample loading is 60-120cm/hr. If a higher load rate is desired, we recommend loading the sample for the first run at a lower flow rate of 60cm/hr, then increase the flow rate on a subsequent runs and compare the results.

Washing

After the sample is applied to the column, the washing cycle begins. The wash buffer may be the equilibration buffer or any other buffer, which suits the application. Adjust the pump flow rate to up to 300cm/hr. Collect approximately 20ml fractions, which help monitor the progress of the washing step. After collecting 6-10 wash fractions (a total of 120-200ml of wash fluid), washing is concluded and elution may begin.

Elution

IgG can be eluted from Protein A Cellthru 300 Plus with Actisep Elution Medium (Sterogene #9701) or any of the standard acidic elution media (pH 2.2-3.5). Generally, we recommend 0.1M Citrate, pH 3.5 as the elution buffer. Alternatively, 2.5M $MgCl_2$, pH 3.5 or 0.1M Glycine, 0.1M NaCl, pH 3.0 can be used.

To preserve the viability of acid-labile IgG, antibodies should be buffer-exchanged within 30 minutes of a low pH elution. Actisep Elution Medium (Sterogene #9701) is recommended to elute antibodies at near neutral conditions. 1M Tris-HCl, pH 7.5 may be added to the eluted fractions until the pH reaches 6.0. This will minimize the pH shock to the antibody. We do not recommend the addition of high pH (9.0) neutralization media.

The recommended elution flow rate is 100 cm/hr.

Cleaning

The resin is cleaned/regenerated between runs with 6 M guanidine, pH 10 or 50% Methanol to remove accumulated lipids and proteins. After regeneration, the resin is washed with deionized water and re-equilibrated with starting buffer. The gel bed may also be cleaned with 0.1 M NaOH, but the 0.1 M NaOH washes must be short (<10 minutes at a time). A total NaOH contact time of 3 hours causes negligible IgG capacity loss.

Store Protein A Ultraflow in 20% ethanol at 2-8^{o}C.