MCLAB

Recombinant Protein G, Agarose

User Manual

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Description

Recombinant Protein G is an immunoglobulin-binding protein derived from the cell wall of the bacterium Staphylococcus aureus. It is composed of five homologous Ig-binding domains that fold into a three-helix bundle. Each domain is able to bind proteins from many of mammalian species, most notably IgGs. Protein A binds with high affinity to human IgG1 and IgG2 as well as mouse IgG2a and IgG2b. Protein G binds with moderate affinity to human IgM, IgA and IgE as well as to mouse IgG3 and IgG1. It does not react with human IgG3 or IgD, nor will it react to mouse IgM, IgA or IgE.

Guidelines

- Use an appropriate bead volume for the amount of IgG sample to be purified
- Column size should be used based on the volume of beads
- A peristaltic pump is recommended for the washing and eluting of IgG
- Wash Buffer and Elution Buffer should be used proportionally to the column bead volume
- Recombinant Protein A agarose should be stored at 2-8ºC

Preparation of Sample

1. Samples containing IgG of interest should be clear and contain no particulates. Filtration or centrifugation should be used if necessary. Delipidation is recommended before IgG purification for samples containing excessive amounts of lipids such as hybridoma supernates and come types of sera.
2. Samples and buffers should be equilibrated to experimental temperature (room temperature or 2-8ºC.
3. pH of samples should be between 6.0 and 7.5. Dilution of sample with Binding Buffer may be necessary to attain desired pH.

Procedure

The following instructions for IgG sample preparation and purification can be scaled up or down depending on user's preference. This manual exemplifies sample preparation from a specific amount of starting material and purification using 1 ml resin.

1. Prepare 1 ml bed volume by loading 2 ml of 50% rProtein G Agarose slurry from supplied bottle onto an empty column (3-5 ml).
2. Equilibrate 1 ml bed volume of rProtein G Agarose with 10 bed volumes of Binding Buffer (10 ml).
3. Load prepared IgG sample onto purification column. (Longer binding time may increase IgG yield).
4. Wash purification column with 6-12 bed volumes (6-12 ml) of Binding Buffer. Unbound proteins should be removed as much as possible. Absorbance of column Wash eluate at A280 should approximate that of Binding Buffer alone after the last wash.
5. Elute IgG of interest with 6 bed volumes (6 ml) of Elution Buffer. Immediately adjust pH of eluate to 7.0 with 0.1 volume (0.6 ml) of 1 M Tris Base.
6. Purification column can be reequilibrated with 4 volumes (4 ml) of Binding Buffer.
7. Purification column may be reused for identical samples starting from step 3 or can be prepared for storage (see Preparation for Storage).

* If there is concern about inactivation of purified IgG due to low pH of Elution Buffer, alternative buffers may be used:

A) 0.5 M Ammonium acetate, pH 3.0
B) 0.1 M Glycine, pH 3.0
C) 0.1 M Sodium citrate, pH 3.0
D) 4 M mgCl2, pH 5.5

Preparation for Storage

1. Once IgG purification is processed, wash purification column with 5 bed volumes (5 ml) of Stripping Buffer.
2. Equilibrate the purification column with 5 bed volumes (5 ml) of Storage Buffer and store at 2-8ºC.

Buffers Preparation

Binding Buffer:
0.15 M NaCl2
0.005 M NaH2PO4
0.005 M Na2HPO4
Adjust pH to 7.0

Elution Buffer
0.1 M Glycine HCl, pH 2.6

Stripping Buffer
1 M Acetic Acid, pH 2.4

Storage Buffer
0.005 M NaH2PO4
0.005 M Na2HPO4
0.15 M NaCl2
0.05% Thimerosal
Adjust pH to 7.0