PureSelect Protein G-Agarose Fast-Flow, 4% Highly Crosslinked

P9302

Storage

Store regenerated Protein G-Agarose in Binding/Wash Buffer containing 20% ethanol at 2-8 °C. Do not freeze.
Stable for 12 months when stored unopened.

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- Product manual
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Introduction

GenDEPOT Protein G-Agarose is an affinity chromatography medium designed for easy, one-step purification of classes, subclasses and fragments of immuno -globulins from biological fluids and from cell culture media. The recombinant protein G ligand is coupled to 4% highly cross-linked agarose. The static binding capacity of Protein G-Agarose is greater than 20 mg sheep IgG/ml settled resin. The dynamic binding capacity will vary depending on several factors such as target antibody, flow rate etc. Protein G, a bacterial cell wall protein isolated from group G Streptococci, binds to mammalian IgGs mainly through Fc regions. Native protein G has 3 IgG binding domains and also sites for albumin and cell -surface binding. The latter have been eliminated from recombinant protein G to reduce nonspecific binding. Although protein G has very similar tertiary structures to protein A, their amino acid compositions differ significantly, resulting in different binding characteristics. Protein G can be used for purification of mammalian monoclonal and polyclonal IgGs that do not bind well to protein A. Protein G has greater affinity than protein A for most mammalian IgGs, especially for certain subclasses including human IgG3, mouse IgG1 and rat IgG2a. Unlike protein A, protein G does not bind to human IgM, IgD and IgA.

Specification

Ligand :	Recombinant Streptococcal protein G lacking the albumin binding sites expressed in E. coli
Binding capacity :	> 20mg sheep IgG/ml settled resin
Resin Volume :	5ml settled resin (10ml 50% slurry)
Bead structure :	4% cross-linked agarose
Bead size range :	90mm (45-165mm)
Storage solution :	1x PBS containing 20% ethanol
Number of IgG binding sites per ligand :	3
M.W. of ligand :	Approximately 22kDa
PI of ligand :	4.69
Degree of Substitution :	Approximately 2mg protein G/ml settled resin

Buffer preparation

Water and chemicals used for buffer preparation should be of high purity. It is recommended filtering the buffers by passing them through a 0.45 µm filter before use.

Binding/Wash Buffer: 20 mM Na_2HPO_4, 0.15 M NaCl, pH 8.0
Elution Buffer: 0.1 M Glycine, pH 2.5
Neutralization Buffer: 1 M Tris-HCl, pH 8.5

Procedure

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

Sample Preparation

To insure that proper ionic strength and pH are maintained for optimal binding, it is necessary to dilute serum samples, ascitic fluid or cell culture supernatant at least 1:1 with Binding/Wash Buffer. Alternatively, the sample may be dialyzed overnight against Binding/Wash Buffer.

Packing of Column

1. Resuspend completely the resin and transfer 1 ml slurry to a new column, in which 1 ml Binding/Wash Buffer was added in advance.
2. Allow the resin to settle down and the buffer to drain from the column.
3. Add 5 ml Binding/Wash Buffer onto the column to equilibrate the resin and drain the buffer with a flow speed of about 1 ml/min.

Column Purification

1. Apply the sample onto the column and drain the flow-through with a flow speed of about 1 ml/min. Collect the flow-through for measuring the binding efficiency to the resin, i.e. by SDS-PAGE.
2. Wash the column with 30 ml Binding/Wash Buffer and drain the buffer with a flow speed of about 2 ml/min, or until the absorbance of the effluent at 280 nm is stable.
3. Elute the immunoglobulins with 10-15 ml Elution Buffer and drain the eluate with a flow speed of about 1 ml/min. Collect the eluate and immediately neutral -ize to pH 7.4 with Neutralization Buffer (1/10 volume of total eluate).

Regeneration of Column

Regenerate the column by washing the resin with 10 ml Elution Buffer followed by equilibration with 5 ml Binding/Wash Buffer. Columns can be regenerated up to 10 times without significant loss of binding capacity.

Related Product

Product Name	Cat No
Puredown Protein G-Agarose	P9202
Puredown Protein A/G-Agarose	P9203
PureSelect Protein A-Agarose	P9301
PureSelect Protein A/G-Agarose	P9303

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