Amintra

Affinty Resins

Protein G

Protein G Affinity Resin
Technical Data and Instruction Manual

Innovators of Protein Technologies

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Introduction

Affinity purification of monoclonal antibodies has been largely confined to the use of Protein A and ProteinG chromatography. The Amintra Protein A and Protein G resins are designed for simple, one-step and rapid antibody purification from serum, ascites and tissue culture supernatant such as those derived from static cultures and bioreactors. Antibody samples purified using this affinity resin may be used in a wide range of laboratory procedures such as 1D or 2D polyacrylamide gel electrophoresis, Western blotting, ELISA etc. The antibodies are sufficiently pure for radiolabelling, conjugations (for example fluorescein) or preparation of immuno-affinity columns.

Storage

The resin is formulated in 20 % ethanol. The Protein G resin as supplied is stable for up to 2 year at 2-8 °C from the date of manufacture. Please see packaging for expiry date. Do not freeze the resin or store it at room temperature. Freezing the suspension will damage the agarose beads. The resin is pre-swollen and de-fined.

Specifications

Protein G	Recombinant Protein G expressed in E. *coli* deficient in albumin binding domain
Supporting matrix:	4% crosslinked agarose resin
Ligand Density:	2 mg Protein G/ml resin
Bead size range:	45-165 µm
Recommended working pH:	pH 2.5- 9.0
Typical binding capacity:	Upto 20 mg Human IgG /ml resin
Linear Flow rate	Upto 300 cm/h (5cm diameter column, pressure 1 bar)
Maximum Flow rate	20-40 ml/min
Optimum Flow Rate	1-10 ml/min
Maximum pressure	0.1MPa (1 bar)
Chemical stability:	High
Solubility in water:	Insoluble
Toxin Levels	Free of Staphyloccocus enterotoxins and hemolysins

Chemical compatibility

All resins are susceptible to oxidative agents. Avoid high temperatures. The resin is resistant to short exposure tof 8M urea, pH 11 and pH 1.0. Protein G is resistant to treatment with 0.1M NaOH.

Disclaimer

This product is for research use only and is not intended for use in clinical diagnosis. No claims beyond replacement of unacceptable material or refund of purchase price shall be allowed.

Recommended Buffers

Binding buffers:

- 10-100 mM Sodium phosphate, 2.7 mM potassium chloride, 0.137 M NaCl pH 7.4 (PBS)
- 50 mM Tris, 2.7 mM potassium chloride,0.137 M NaCl pH 8.0 (TBS)
- 20-100 mM Sodium phosphate pH 7.0-7.2
- 20 mM Sodium acetate pH 7.4

Elution buffer:

- 0.1 M Sodium citrate pH 3.0-6.0
- 0.1-0.2 M Glycine/HCl pH 2.5-3.0
- 0.1 M Sodium acetate pH 3.5 pH 3.0-6.0

Neutralization buffer:

- 1M Tris/HCl pH 9.0

Protein Purification Protocol

Delipidation Procedure:

All protein A & G affinity columns are affected by the presence of lipids and lipoproteins, especially in antibody samples derived from ascites fluid. For end users who have antibody solutions which they need to delipidate, the following protocol is a gentle and easy method for removing lipids and lipoproteins.

1) Add 0.04 ml 10% dextran sulphate solution and 1 ml 1 M calcium chloride per ml sample.

2) Mix for 15 min.

3) Centrifuge at 10,000 g for 10 min.

4) Discard the precipitate.

5) Exchange the sample into TBS (Tris Buffered Saline) using dialysis, ultrafiltration or a desalting column. **Do not buffer exchange into a phosphate-containing buffer such as PBS**.

Pre-equilibration:

Equilibrate the resin with 3-5 column volumes of binding buffer.

Sample loading:
Load an appropriate amount of 0.45 μm filtered cleared lysate on to column or mix the resin with the cleared lysate and let the resin settle for 30 minutes at room temperature or at 2-8 °C. The binding capacity of the resin is approximately 20-30 mg IgG/ml sedimented resin. Collect the sample flowthrough for further analysis.

Washing:

Wash the column 5x with 3 column volumes of wash buffer. Collect the washes for further analysis to ensure that all unbound protein is removed.

Elution:

Apply 10-15 column volumes elution buffer to the resin and collect appropriate fractions sizes (e.g 1CV) for further analysis.

Alternatively you can apply 2 column volumes of elution buffer to the resin, mix and allow the resin to settle. Following this, collect the supernatant. Repeat this process at least 5 times.

The eluate must be neutralized rapidly. You can either elute the purified IgG directly into a high pH neutralization buffer eg 1M Tris/HCl pH 9.0 or dialyse. Always check the protein content of each fraction before pooling to avoid unnecessary dilution of the purified target protein.

Regeneration:

Wash the column with 10 CVs of elution buffer followed by 10 CVs of binding buffer. Proceed to the pre-equilibration step of another bind-wash-elute cycle if the column is to be re-used immediately. After regeneration, the resin can also be stored in a screw capped bottle in 0.1 % sodium azide (made up in distilled water) at 2-8 °C until further use.

Detemine Antibody Concentration

For pure solution the Beer-Lambert law, A = ε.c.l, can be used to determine the protein concentration of IgG (mg/ml).

	Extinction Coefficient ($ml.mg^{-1}.cm^{-1}$)
IgG	0.72
IgM	0.84
IgA	0.94

Sandwich ELISA assay can also be used to accurately measure antibody concentrations within a range of 1 mg/ml to 20 mg/ml sample. The antibodies can also be monitored for purity by SDS-PAGE under reducing or non-reducing conditions. Note that IgG appears in a reducing SDS-PAGE as 25 kDa and 50-55 kDa bands and IgM appears as 25 kDa and 70-80 kDa bands. Recovery of immunoglobulins can be quantified by a standard protein assay, scanning densitometry of reducing or non-reducing SDS-polyacrylamide gels or ELISA. Antigen binding parameters can be measured for both affinity and

Ordering Information

Cat #	Description
APG0005	Amintra Protein G – 5ml
APG0025	Amintra Protein G – 25ml
APG0100	Amintra Protein G – 100ml

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