Version 2b Last updated 9 September 2020

ab270309 Protein G Resin - Amintra

A product of Expedeon, an Abcam company

Applicable to Expedeon product codes APG0005, APG0025, APG0100.

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Protein G Resin - Amintra datasheet:
www.abcam.com/ab270309
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For simple, rapid purification of classes, subclasses and fragments of immunoglobulins from biological fluids and cell culture media.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Protein G Resin - Amintra (ab270309) is an affinity chromatography medium designed for easy, one-step purification of classes, subclasses and fragments of immunoglobulins from biological fluids and from cell culture media. The recombinant protein G ligand is coupled to highly cross-linked 4% agarose. The binding capacity of Protein G Resin - Amintra is greater than 30 mg human IgG/mL medium. With the high binding capacity and chemical stability, very high batch-to-batch reproducibility, which ensures excellent performance on isolation of immune complexes.

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 recombinant protein G have been eliminated the nonspecific binding site. 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 IgG_3, mouse IgG_1 and rat IgG_{2a}. Unlike protein A, protein G does not bind to human IgM, IgD and IgA.

2. Materials Supplied and Storage

Store kit at +4°C immediately on receipt. **Do not freeze or store the resin at room temperature. Freezing the suspension will damage the agarose beads.** The resin is pre-swollen and defined. It is formulated as a 50% slurry containing 20% ethanol.

Item	Quantity			Storage temperature
Protein G Resin - Amintra	5 mL	25 mL	100 mL	+4°C

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Binding buffer (see buffer selection Section 4.1)
- Wash buffer (see buffer selection Section 4.1)
- Elution buffer (see buffer selection Section 4.1)
- Neutralization buffer (see buffer selection Section 4.1)

4. Technical Considerations

4.1 Recommended buffers:

Water and chemicals used for buffer preparation should be of high purity. We recommend filtering the buffers by passing them through a 0.22 µm or 0.45 µm filter before use.

Binding buffer/wash : 20 mM NaH_2PO_4, 150 mM NaCl, pH 7.0
Elution buffer: 100 mM glycine, pH 3.0
Neutralization buffer: 1 M Tris-HCl, pH 8.5

4.2 Characterization of the Resin:

Supporting matrix	Highly cross-linked 4% agarose supplied as 50% slurry
Ligand	Recombinant Protein G
Bead size range	45-165 µm
pH stability	long term: pH 3 – 9 short term: pH 2 - 10
Typical binding capacity	>30 mg Human IgG/mL resin
Maximum pressure	0.3 MPa (3 bar)
Chemical stability	High - The IgG binding capacity and recovery was maintained after storage for: 1. 7 days at 37°C in: • 1 M acetic acid pH 2.0, 20 mM sodium phosphate, 1% SDS, pH 7.0 • 6 M Guandine-HCl, pH 7.0 • 70% ethanol. 2. 2 hours at room temperature in: • 0.1 M HCl, pH 1.0 • 8 M urea, pH 10.5 • 0.1 M Glycine-NaOH, pH 11
Storage in water	Insoluble

4.3 Protein A and Protein G antibody binding affinities:

Species	Subclass	Protein A Binding Affinity	Protein G Binding Affinity
Human	IgA	variable	-
	IgD	-	-
	IgE	-	-
	IgG1	++++	++++
	IgG2	++++	++++
	IgG3	-	++++
	IgG4	++++	++++
	IgM	variable	-
Avian egg yolk	IgY	-	-
Cow		++	++++
Dog		++	+
Goat		-	++
Guinea pig	IgG1	++++	++
	IgG2	++++	++
Hamster		+	++
Horse		++	++++
Koala		-	+
Llama		-	+
Monkey (Rhesus)		++++	++++
Mouse	IgG1	+	++++
	IgG2a	++++	++++
	IgG2b	+++	+++
	IgG3	++	+++
	IgM	variable	-
Pig		+++	+++
Rabbit	No distinction	++++	+++
Rat	IgG1	-	+
	IgG2a	-	++++
	IgG2b	-	++
	IgG3	+	++
Sheep		+/-	++

++++ Strong binding; ++ Medium binding; - Weak or no binding

4.4 Imporving binding conditions:

Any sample, such as a crude biological extract, a cell culture supernatant, serum, ascites or an artificial standard can be used with the Protein A resin. Aggregation/precipitation of proteins is common during storage and repeated freeze/thaw cycles of sera, ascites and tissue culture supernatants. It is important that the sample is first filtered through a 0.45 - 1.2 µm filter to remove particulates that could clog the resin flow channels. **All samples should be filtered just prior to loading** even if they have been filtered several days before the chromatographic run.

Lipids, which can be found at high levels in serum or ascites should also be removed as much as is possible prior to loading, please refer to our recommended Delipidation Protocol in step 5.1. Of equal importance is the ability to process the samples rapidly and, if the need arises, to be able to purify the target protein at 4˚C.

5. Assay Procedure

5.1 Delipidation protocol:

All protein 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:

5.1.1 Add 0.04 mL 10% dextran sulphate solution and 1 mL 1 M calcium chloride per mL sample.

5.1.2 Mix for 15 minutes.

5.1.3 Centrifuge at 10,000 x *g* for 10 minutes.

5.1.4 Discard the precipitate.

5.1.5 Exchange the sample into Tris-buffered saline (TBS) using dialysis, ultrafiltration or a desalting column.

Δ Note: Do NOT buffer exchange into a phosphate-containing buffer such as PBS.

5.2 Standard purification protocol:

5.2.1 Pre-Equilibration:

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

5.2.2 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 flow-through for further analysis.

5.2.3 Washing:

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

5.2.4 Elution:

Apply 10-15 column volumes elution buffer to the resin and collect appropriate fractions sizes (e.g 1 CV) 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 e.g. 1M Tris-HCl, pH 8.5 or dialyse.

5.2.5 Always check the protein content of each fraction before pooling to avoid unnecessary dilution of the purified target protein.

5.2.6 Regeneration:

In general, Protein G Resin - Amintra are well suited for reuse a number of times. When precipitation and protein aggregation cause the loss of velocity and combined loads, you need to clean the medium.

To remove the precipitation or denatured protein: Wash the column with 2 column volumes 6M guanidine hydrochloride solution. Finally wash the column with 5 column volumes 1X PBS (pH 7.4).

To remove the non-specific adsorption protein: Wash the column with 3 column volumes 70% ethanol or 1% Triton X-100. Finally wash the column with 5 column volumes 1X PBS (pH 7.4).

5.3 Packing the column for use with an AKTA system:

Column packing:

5.3.1 Remove air from the column dead spaces by flushing the end-piece and adapter with packing buffer. Make sure no air has been trapped under the column net.

5.3.2 Close the column outlet leaving the net covered with packing buffer.

5.3.3 Resuspend the resin medium in its container by shaking (avoid stirring the sedimented medium). Pouring the slurry down a glass rod held against the column wall will minimize the introduction of air bubbles.

If using a packing reservoir, immediately fill the remainder of the column and reservoir with packing buffer. Mount the adapter or lid of the packing reservoir and connect the column to a pump. Avoid trapping air bubbles under the adapter or in the inlet tubing.

5.3.4 Open the bottom outlet of the column and set the pump to run at the desired flow velocity. Ideally, Amintra Protein G is packed at a constant pressure of approximately 1 bar (0.1MPa). If the packing equipment does not include a pressure gauge, use a packing flow velocity of approximately 400 cm/h (10 cm bed height, 25°C, low viscosity buffer).If the recommended pressure or flow velocity cannot be obtained, use the maximum flow velocity the pump can deliver. This should also give a reasonable well-packed bed. Do not exceed 75% of the packing flow velocity in subsequent chromatographic procedures.

5.3.5 When the bed has stabilized, close the bottom outlet and stop the pump.

If using a packing reservoir, disconnect the reservoir and fit the adapter to the column. If using the column, carefully place the top filter on top of the bed before fitting the adapter.

5.3.6 With the adapter inlet disconnected, push the adapter down, approximately 2 mm into the bed, allowing the packing solution to flush the adapter inlet.

5.3.7 With the adapter inlet disconnected, push the adapter down, approximately 2 mm into the bed, allowing the packing solution to flush the adapter inlet.

5.3.8 Connect the pump, open the bottom outlet and continue packing. The bed will be further compressed at this point and a space will be formed between the bed surface and the adapter.

5.3.9 Close the bottom outlet. Disconnect the column inlet and lower the adapter approximately 2 mm into the bed. Connect the pump. The column is now ready to use.

Sample Purification protocol:

5.3.10 Fill the syringe or pump tubing with binding buffer. Remove the stopper and connect the column to the syringe (with the provided connector), or pump tubing, "drop to drop" to avoid introducing air into the column. Remove the snap-off end at the column outlet.

5.3.11 Wash the column with 10 column volumes of binding buffer.

5.3.12 Apply the sample, using a syringe fitted to the connector or by pumping it onto the column.

5.3.13 Wash with 5 to 10 column volumes of binding buffer or until no material appears in the effluent.

5.3.14 Elute with 5 column volumes of elution buffer. Other volumes may be required if the interaction is difficult to break

Analysis:

5.3.15 Identify fractions using UV absorbance, SDS-PAGE, or the Western blot. We recommend our RunBlue™ range of precast gels.

Bradford assay:

For determination of antibody concentrations using the Bradford assay, it is recommended that a standard curve be prepared using known samples of pure antibody.

UV absorbance:

For pure solution rearranging the Beer-Lambert law, $A = \varepsilon.c.l$, can be used to determine the protein concentration of IgG (mg/mL). A = Absorbance at 280 nm, ε = Extinction Coefficient, c = Concentration of Sample (mg/mL) and l = path length (cm).

Immunoglobulin	Extinction Coefficients ($mL.mh^{-1}.cm^{-1}$)
IgG	0.72
IgM	0.84

IgA	0.94

ELISA and SDS-PAGE:

Sandwich ELISA assay can also be used to accurately measure antibody concentrations within a range of 1 mg/mL to 20 mg/mL sample. Antibody samples 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-PAGE gels or ELISA.

Regeneration:

5.3.16 In general, Protein G Resin - Amintra are well suited for reuse a number of times. When precipitation and protein aggregation cause the loss of velocity and combined loads, you need to clean the medium.

To remove precipitation or denatured protein: Wash the column with 2 column volumes 6M guanidine hydrochloride solution. Finally wash the column with 5 column volumes 1X PBS (pH 7.4).

To remove non-specifically absorbed proteins: Wash the column with 3 column volumes 70% ethanol or 1% Triton X-100. Finally wash the column with 5 column volumes 1X PBS (pH 7.4).

5.4 Resin storage:

Store Protein G Resin - Amintra in 1X PBS containing 20% ethanol at 2°C to 8°C. **Do not freeze.**

6. Troubleshooting

6.1 Back pressure exceeds 1 bar.

6.1.1 Column might be clogged. Please follow protocol for "Regeneration"

6.1.2 Sample solution might contain a precipitate. Filter the sample solution by passing them through a 0.22 µm or 0.45 µm filter.

6.2 The flow rate is very low.

6.2.1 Tiny air bubbles from buffer or particles from the sample are blocking the gel pores. De-gas buffers and samples. Do not allow the column to dry.

6.3 No antibody is detected in any elution fraction.

6.3.1 The concentration of antibody of interest is very low. Purify the antibody using the specific antigen coupled to a beads.

6.3.2 The antibody is sensitive to low-pH elution buffer. Neutralize the eluted fractions with Neutralization Buffer immediately after elution.

6.3.3 The IgG subclass does not bind to Protein G. Try other affinity chromatography media to purify the antibody, such as media conjugated with Amintra Protein A.

Technical Support

For all technical or commercial enquiries please go to:

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