Amintra™ Protein A Affinity Resin

expedeon

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ORDERING INFORMATION

PRODUCT	SIZE	CAT. NO.
Amintra™ Protein A	5 ml	APA0005
Amintra™ Protein A	25 ml	APA0025
Amintra™ Protein A	100 ml	APA0100
Amintra™ Protein A	Custom Volume	APACUST

DESCRIPTION

Amintra™ Protein A Affinity Resin 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 A ligand is coupled to highly cross-linked 4% agarose resin. The coupling is optimized to give high binding capacity for immunoglobulins. The static binding capacity of Amintra Protein A Affinity Resin is greater than 40 mg human IgG per mL settled resin. The dynamic binding capacity varies depending on several factors such as the target antibody, flow rate during binding, etc.

Protein A, a bacterial cell wall protein isolated from Staphylococcus aureus, binds to mammalian IgGs mainly through Fc regions. Recombinant protein A contains five high affinity IgG binding domains with other non-essential domains removed to reduce nonspecific binding. Since only the Fc region is involved in binding, the Fab region is available for binding antigens.

STORAGE

The Protein A 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.

SPECIFICATION

Supporting matrix	Highly cross-linked 4% agarose supplied as 50% slurry
Ligand	Recombinant Protein A
Bead size range	45-165 µm
Recommended working pH	pH 2.5-9.0
Typical binding capacity	>40 mg Human IgG /ml resin
Maximum Flow rate	Up To 300 cm/h
Maximum pressure	0.3MPa (3 bar)
Chemical stability	High - Stable in all aqueous buffers commonly used in Protein A chromatography: • 10 mM HCl (pH 2) • 1 mM NaOH (pH 11) • 0.1 M sodium citrate/HCl (pH 3) • 6 M guanidine-HCl • 20% ethanol
Storage buffer	1x PBS containing 20% ethanol

PROTEIN A CHROMATOGRAPHY

Protein A is a cell wall protein from Staphylococcus aureus with a molecular weight between 35-50 kDa. Immobilized Protein A resins linked via an amide bond between the amino groups of protein A and either oxirane or Nhydroxysuccinimide ester groups form the most stable cross-links. Immobilized Protein A binds specifically to the Fc region of immunoglobulin molecules of many mammalian species.

Protein A affinity chromatography is a rapid one-step purification, which removes most nonIgG contaminants and can achieve purities close to homogeneity. It is particularly useful for purifications of tissue culture supernatant.

Improving 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. When protein aggregation or protein solubility problems are observed, Expedeon's Stabil-PAC (# STP) product can be used the enhance protein stability and solubility.

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 on Page 6. 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.

Optimal conditions for binding the target molecule to a resin are critical for successful separation of the protein. If the binding conditions are not optimal with respect to pH, salt concentration, presence or absence of metal ions etc, the interaction could be weak or nonexistent. In many instances, the sample needs to be dialysed or diafiltered by ultrafiltration before it is applied to an affinity or ion exchange chromatographic support. In Protein A separations, the sample should simply be diluted 1:1 (v/v) in 1 x binding buffer.

The interaction of immobilized Protein A with immunoglobulins (Igs) is pH dependent. The binding capacity for Protein A is optimal at pH 8-9. Salt concentration can significantly affect the binding of mouse Igs to protein A. Mouse IgG1, rat IgG1 and rat IgG2b bind well to immobilized protein A when the salt concentration is higher than 1 M, but bind poorly at low salt concentrations.

Protein Elution

The most common elution conditions for Protein A affinity and immuno-affinity separations involve a reduction in pH to between pH 2.5 and 5.0. It is important to appreciate that a few proteins (e.g. some monoclonal antibodies) are acid-labile and they can lose their activity at very low pH values. Above all, the elution conditions must preserve the integrity and activity of the target protein. Most observed denaturation is caused by harsh elution conditions. Acidic pH is known to reduce the antibody titre, decrease immunoreactivity and distort the antibody structure. It is therefore critical that the pH is restored to neutrality after elution.

Flow rate is an important consideration for achieving optimal separation. Flow rate through the column support is related to the efficiency of the separation; too fast and the mobile phase will move past the beads quicker than the diffusion time necessary for the antibodies to reach the internal bead volume. This will also result in lower apparent binding capacity for the resin. Amintra Protein A resin chemistry provides very rapid association kinetics between the protein molecule and the immobilized ligand, giving optimal diffusional flow through the internal bead structure and allowing the use of high flow rates.

Protein A or Protein G

Immunoglobulin G from most species consists of several subclasses with different biological properties. Four subclasses of IgG have been identified in human (IgG1, IgG2, IgG3, and IgG4) and in mouse (IgG1, IgG2a, IgG2b and IgG3). For immunological studies, it is often necessary to isolate one particular subclass of IgG from the other subclasses.

Protein G binds to all major Ig classes except IgM and therefore has a wider reactivity profile than Protein A. However, the binding of Igs to Protein G is often stronger, requiring more stringent elution conditions for complete recovery of the immunoglobulin compared to Protein A.

Protein A can withstand more harsh conditions which can be beneficial for deep cleaning and regeneration. Different mouse IgG subclasses will exhibit varying strength of association to Protein A. **Customization of the purification strategy may be required for the affinity separation**, e.g. mouse IgG1, the most common subclass used, does not bind well to Protein A at low ionic strength. However, the use of high salt concentrations (2-3 M NaCl) and high pH (pH 8-9), these antibodies will bind to Protein A and provide good separation.

The needs of the researcher dictate that the speed of sample processing, the cost and the reproducibility are key criteria for selecting purification tools. Amintra purification resins have been designed to offer the optimal solution to each criterion. In the vast majority of cases, simply selecting the correct resin and performing a considered purification strategy will provide the best possible separation of your target proteins.

Recommended Buffers

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

Binding/Wash buffer:

- 0.15 M NaCl, 20 mM Na_2HPO_4, pH 7.0

Elution buffer:

- 0.1 M Glycine, pH 3.0

Neutralization buffer:

- 1M Tris-HCl, pH 8.5

Protein Purification Protocol

Delipidation Procedure:
All protein A affinity columns are affected by the presence of lipids and lipoproteins, which are especially common 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.

Standard Protocol:

1. Pre-equilibration:
   Equilibrate the resin with 3-5 column volumes of binding buffer.

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 >40 mg IgG/ml sedimented resin. Collect the sample flowthrough for further analysis.

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.

4. 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 e.g. 1M Tris-HCl, pH 8.5 or dialyse. Always check the protein content of each fraction before pooling to avoid unnecessary dilution of the purified target protein.

5. Regeneration:
   Wash the column with 10 CVs of elution buffer followed by 5 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. Columns can be regenerated up to 10 times without significant loss of binding capacity.

Packing the column for use with an AKTA system

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

- Open the bottom outlet of the column and set the pump to run at the desired flow velocity. Ideally, Amintra Protein A 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.
- 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.

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

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

- 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.
- Wash the column with 10 column volumes of binding buffer.
- Apply the sample, using a syringe fitted to the connector or by pumping it onto the column.
- Wash with 5 to 10 column volumes of binding buffer or until no material appears in the effluent.
- Elute with 5 column volumes of elution buffer. Other volumes may be required if the interaction is difficult to break.

Analysis

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

Regeneration

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.

Storage

Store Amintra Protein A medium in Binding/Wash Buffer containing 20% ethanol at 2°C to 8°C. **Do not freeze.**

Determination of Antibody Concentration

By Bradford Assay
Expedeon's BradfordUltra assay provides a robust assay for the determination of protein concentrations from 1 µg/ml to 1.5 mg/ml. For determination of antibody concentrations using BradfordUltra, it is recommended that a standard curve be prepared using known samples of pure antibody.

By UV Absorbance
For pure solution rearranging the Beer-Lambert law, A = ε.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)

	Extinction Coefficient ($ml.mh^{-1}.cm^{-1}$)
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.

Troubleshooting

The flow rate of the column is very low.

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

A considerable amount of sample has been loaded, but no specific antibody of interest is detected.

The concentration of antibody of interest is probably very low. Purify the antibody using the specific antigen coupled to a support matrix (eg. Amintra NHS-activated beads, Cat. # AMS0025 or AMS0100).

The antibody is degraded.

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

No antibody is detected in any elution fraction.

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

Related Products

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		12%		NXG01212	NXG01227
		16%		NXG01612	NXG01627
		4-8% (Gradient)		NXG40812	NXG40827
		4-12% (Gradient)		NXG41212	NXG41227
		4-20% (Gradient)	NXG42002	NXG42012	NXG42027
Teo-Tricine SDS	8 x 10 cm cassette Compatible with BioRad tanks	8%		BCG00812	BCG00827
		10%		BCG01012	BCG01027
		12%		BCG01212	BCG01227
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Bis-Tris	10 x 10 cm cassette Compatible with SureLock™ tanks	8%	NBT00812	NBT00827
		10%	NBT01012	NBT01027
		12%	NBT01212	NBT01227
		4-12% (Gradient)	NBT41212	NBT41227

BFU05L	BradfordUltra
STP	Stabil-PAC (Contains NVoy Polymer + Release Agents)
DCX-700	Dual Cool Mini-Vertical System
EPS-300 X	Power Supply, 1-300V, 1-500mA

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