

Affinity support for the purification of IgG antibodies

rProtein A-Affarose

Product Description

Name :	Recombinant Protein A immobilized onto agarose gel			
Catalog Number :	UP49981G	2ml of gel	UP49981H	5ml of gel
Matrix :	modified Agarose 6%			
Binding capacity :	Approx. 20 mg of human IgG per ml of wet gel			
Stability :	Lowest leaching <3ng rProtein /mL. Drug Master File			
Packaging :	Protein A Affarose is supplied in solution at 50% with Thimerosal 0,01%			
Storage :	+4°C. Stable for a minimum of 1 year from date of receipt.			

Technical Information

Uptima offers high quality proteinA-affarose for various in vitro use R&D technics and applications :

- Purification of IgGs from serum, ascites and hybridoma supernatant
- Immunoprecipitation technics, IgG immobilization, affinity columns preparation, preadsorbition, IgG depletion...

- Protein A is a highly stable surface receptor of 42 kDa produced by *Staphylococcus aureus*, which is capable of binding the Fc portion of immunoglobulins, especially IgGs, from a large number of species (Boyle, 1987). Each protein A molecule can bind 2 molecules of IgG, allowing the formation of a precipitate (Sjöholm, 1975).
- Protein A binds human IgG subclasses, IgM (medium), IgA and IgE ; and mouse IgG1 (weakly), IgG2a and IgG2b. Protein A also binds IgGs from various other laboratory and domestic animals (+++/Rb, Mo, Pg, Dg, Ct, Ha, Gp, ++/Dk), but predominantly only isotypes from some animals (IgG2/Sh, Bv, Gt; IgG2c/Rt). Ask for [known specificities](#).
- The matrice is a 6% highly crosslinked modified agarose designed for optimal results in affinity purifications thanks to its hydrophilicity and flow characteristics.
- rProteinA is crosslinked to agarose to ensure high chemical and mechanical stability. The binding capacity is 18-22mg of human IgG per ml of wet gel, but may differ from isotype to isotype and from species to species.
- The gel can be used in batch or in packed columns. Batch is convenient for analytical separations (immunoprecipitation) from different and complex samples, while columns are preferred for repeated uses. Exceptional consistency in manufacturing process suits to industrial applications

For use *in vitro* only, not for diagnostic.

Related documents and products

[FT-UP75196](#)
[FT-UP52746](#)

ProteinG-affarose
ProteinL-affarose

[protein G](#)
[protein L](#)
[FT-UP40290](#)

Protein A

NT-40290

For any question,
contact your local distributor

Uptima,
powered by



213 Avenue J.F. Kennedy - BP 1140
03103 Montluçon Cedex - France
Tél. 04 70 03 88 55 - Fax 04 70 03 82 60

uptima@interchim.com Hotline : 33(0)4 70 03 76 06

Directions for Use

Guidelines for purification and immunoprecipitation

The sample from which you want to purify IgG, may be serum, ascite, hybridoma culture supernatant, or other biological fluids. Attention should be paid to remove insolubles (by filtration), and buffer exchange may be needed. If IgG are purified from in vitro mixture like protein extracts, 150-500 mM NaCl, 20 mM phosphate, SDS, 0.1-1% Nonidet40, 0.1-1% DOC and protease inhibitors may have been used for cell lysis or solubilization steps. Detergents, notably, should be removed prior to the protein A interaction step, i.e. by gel filtration.

All or some purification steps can be performed in batch, or in packed columns for better convenience / performance. Below is a protocol recommended for the purification from serum.

In batch procedure, antibody binding is slightly more favorable, but gel washing is less efficient and eluted antibodies are recovered more diluted with lower yields. The gel can be sedimented under ca 1000g centrifugation, and supernatant removed.

Column purification requires a column with 20µm frits. Circulation by gravity is not recommended because of a too low flow rate : use a peristaltic pump or a FPLC system with suitable tubes and fittings (ask Uptima).

Recommended Column / Samples Volumes:

Biological Fluids	1 ml of gel will bind (ml of sample)
Immune Serum	0.5 ml
Supernatant (+10% FCS)	5 ml
Supernatant (serum-free)	100 ml
Ascites Fluid	0.5 ml

Protocol for IgG purification from serum

- Incubate ProteinA-afarose with 0.45 µm filtrated serum, 4 hours under constant agitation (batch) or under circulation (column) at room temperature.
Notes : It may be necessary to first delipidated certain sera. Dilution with IgG Loading and Wash Buffer #UPQ99541 may improve IgG binding. Longer incubation time may also to achieve higher yields, but lower affinity antibodies could be purified. For sensitive antibodies, incubation may be performed overnight at +4°C.
- Wash the gel with IgG Loading and Wash Buffer #UPQ99541 or PBS (150-500 mM NaCl, 20 mM phosphate, pH 7.5, #UP30715 (tabs) or UP68723 (powder)). Wash until of unbound molecules are completely removed (monitor optical absorbance, until OD280 nm < 0.05).
- Elute bound antibodies from the gel with IgG Elution Buffer #UPQ99542 (or citric or Acetic acid 0.1 M pH 3) under constant circulation. Eluted fractions should be neutralized rapidly with IgG Neutralizing Buffer UPQ99543 (or with Tris 1 M pH 9.0) to prevent the degradation of the purified antibodies. Also ask for our mild IgG elution buffer #UP38591.
- The purified fraction could be desalted by dialysis or other means (ask Uptima CelluSep and FastDialyser), and analysed as desired.
- Reequilibrate the column with PBS. Store protein A - affarose in PBS + 20% ethanol at +4°C.

Other Information

For any information, please contact Uptima, or your local distributor.

213 av.J.F.Kennedy, 03103 Montlucon, fax : +33(0)4 70 03 82 60, hotline Interbiotech : +33(0)4 70 03 73 06

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Uptima,
powered by



213 Avenue J.F. Kennedy - BP 1140
03103 Montluçon Cedex - France
Tél. 04 70 03 88 55 - Fax 04 70 03 82 60

uptima@interchim.com Hotline : 33(0)4 70 03 76 06