FT-UP90467

Affinity support for the purification of IgG antibodies

rProtein A-Affarose XTreme

Features

Name:	**Recombinant Protein A** immobilized onto agarose, highest available binding capacity
Catalog Number:	UP904670, 5 ml of gel UP904671, 20 ml of gel Inquire for other quantities
Matrix:	Agarose 6%
Ligand:	Recombinant protein A (no animal contact, endotoxin free)
Binding capacity:	Approx. **40 mg of human IgG** per ml of wet gel (static)
Leaching:	< 5 ng proteinA /ml of gel
Max flow rate:	400 cm/hr
Recommended working flow rate :	30-300 cm/hr
Packaging :	Protein A Affarose XTreme is supplied in solution at 50% with Thimerosal 0,01%
Storage:	+4°C (L) . Stable for a minimum of 1 year from date of receipt.

Scientific and Technical Information

Uptima offers this proteinA-affarose reagent for various R&D immobilization of IgG applications *in vitro use* :

- immunoprecipitation technics before 1D or 2D electrophoresis, western-blotting, ELISA analysis…
- purification of polyclonal IgGs from serum
- purification of monoclonal IgGs from ascites, hybridoma supernatant, bioreactor cultures…
- antibody concentration, desalting, endotoxin removal…
- isotype differentiation
- preparation of affinity columns

- Protein A properties and very simple-use efficiency, make it the most attractive and popular support for Ig purifications ; bind, wash, and elute with appropriate buffers.
- Protein A (#40290A) 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 the table of known specificities.
  Uptima Protein A is produced by recombinant technology in E.coli, culture media being exempt of any animal proteins, to give by highly controled procedure devoided of toxic bacteral contaminant found normally in native Protein A (Free of Staphyloccocus endotoxins and hemolysin).

For any question,
contact your local distributor

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- The gel is a 6% crosslinked and modified agarose optimized for affinity purification thanks to its hydrophilicity and flow properties.
- rProteinA is covalently link to agarose, (3.5mg/ml). The capacity of IgG binding is >30mg of human IgG per ml of wet gel. Note : capacity may differ from isotype to isotype, from species to species, and depends also on binding/elution conditions. Uptima recommends the use from pH 3 to pH 10 (pH :2-11 for cleaning). The covalent coupling is very stable, ensuring minimal leaching of ligand (<5ng/ml, measured by ELISA), and excellent stability (keep >95% binding capacity over 32 runs). Cleaning in place may be performed with NaOH (>85% binding capacity over 100 cycles). Protein A resist to 6 M guanidin HCl, 8 M Urea, and 2 M sodium thiocyanate. However, the support is sensitive to oxidative agents and high temperatures.
- The gel can be used in batch, in packed columns, or in immunoprecipitations devices. Batch is convenient for analytical separations (immunoprecipitation) from different and complex samples, while columns are preferred for repeated uses. It suits to GMP productions that require harsh treatment e.g. peroxyacetic acid.

Species of Igs	Protein A binding immunoglobulin +++ strong ++ medium	+ weak +- contradictory datas	? not available - no binding
Human	+++/ IgG (IgG1, IgG2, IgG4) ++ / IgM, IgE, IgD, IgA		IG3
Mouse	+++/ IgG (, IgG2a, ++ / IgG2b, IgG3),	IgG1, IgM	
Rat		IgG2c, IgM	IgG1, IgG2a, IgG2b
Rabbit	+++ / IgG		
Pig	+++/ IgG		
Guinea Pig	IgG		?/ IgG
Hamster		IgG	?/ IgG
Goat			-/ IgG (IgG1, IgG2)
Sheep		+-	-/ IgG (IgG1, IgG2)
Bovine / Cow		IgG	-/ IgG (IgG1, IgG2)
Chicken			- / IgY
Human Fab		+	
Human F(ab')2		+	
Human scFv		+	
Human Fc	++		
Human κ			-
Human λ			-

Directions for use

Guidelines for buffers
(see below for example of buffers to use in serum, ascite or supernatant purification)

Binding Buffer : Moderatly alcaline buffers (pH 7.5-9). PBS functions adequately for IgG.

Elution Buffer : Low pH buffers are generally required for antibody elution. Human IgG's generally require lower pH buffers for elution compared to other species. Examples are:

Murine IgG – 0.1M Sodium Citrate (pH 3.0)
Human IgG – 0.2M Glycine (pH 2.0)

Neutralizing Buffer Sensitive antibodies may require the elution pool be neutralized after exposure to low pH – 1M Tris-HCl (pH 8.5).

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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 payed to remove insolubles by 0.45 µm filtration just before purification.
Buffer exchange may be needed (dilution 1/1 with binding buffer).
Extracts may have been obtained with a formulation like 150-500 mM NaCl, 20 mM phosphate, SDS, 0.1-1% Nonidet 40, 0.1-1 % DOC, and protease inhibitors.
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 <1000g centrifugation, and supernatant removed.
Column purification requires a column with 20µm frits. Use a peristaltic pump or a FPLC system with suitable tubes and fittings to speed up the procedure (ask Uptima).

Protocol for IgG purification from serum

- Equilibrate the Protein A with 10 volumes of Binding Buffer.
- Centrifugate the serum 10 000g for 10 min ; filtrate on 0.45µm. It may be necessary to first delipidated (cf paragraph "delipidation protocol"). Dilute the sample 1:1 in Binding Buffer.
- Incubate ProteinA-affarose with 0.45µm filtrated serum, 4 hours under constant agitation (batch) or under circulation (column) at room temperature.
  Notes : to achieve higher yields longer incubation time is necessary, but lower affinity antibodies could be purified. For sensitive antibodies, incubation may be performed overnight at +4°C.
- Wash the gel with Binding Buffer. Wash until unbound molecules are completely removed (monitor optical absorbance, until OD280nm <0.05).
- Elute bound antibodies from the gel with IgG Elution Buffer 2 (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 1M pH9.0) 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.
- Equilibrate the column with PBS. Store protein A - affarose in PBS + 20% ethanol at +4°C.

Uptima recommends the following buffer :

Binding Buffer	1.5M glycine, 3M NaCl, pH9
Elution Buffer1	0.1M sodium citrate buffer pH5.5
Elution Buffer2	0.2M citrate buffer pH2.5 (or 0.1M Citric Acid pH3.0)
Neutralizing Buffer	1.0 M Tris-HCl, pH 9.0

Protocol for IgG purification from ascite and supernatant

- Equilibrate the Protein A with 10 volumes of Binding Buffer.
- Centrifuge the ascite 10 000 g for 10 min. Filtrate the ascite or supernatant on 0.45µm. It may be necessary to first delipidated the ascite (cf paragraph "delipidation protocol"). Dilute the sample 1:1 in Binding Buffer.
- Incubate ProteinA-affarose with 0.45µm filtrated sample, 4 hours under constant agitation (batch) or under circulation (column) at room temperature.
  Notes : to achieve higher yields longer incubation time is necessary, but lower affinity antibodies could be purified. For sensitive antibodies, incubation may be performed overnight at +4°C.

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- Wash the gel with Binding Buffer. Wash until unbound molecules are completely removed (monitor optical absorbance, until OD280nm <0.05).
- Elute bound antibodies from the gel according with the two following steps:
  - Elute with IgG Elution Buffer 1 under constant circulation. Eluted fractions should be neutralized rapidly with IgG Neutralizing Buffer UPQ99543 (or with Tris 1M pH9.0) to prevent the degradation of the purified antibodies. Stop the elution when OD280nm <0.05.
  - Elute with IgG Elution Buffer 2 under constant circulation. Eluted fractions should be neutralized rapidly with IgG Neutralizing Buffer UPQ99543 (or with Tris 1M pH9.0) to prevent the degradation of the purified antibodies. Stop the elution when OD280nm <0.05.
- 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.

Uptima recommends the following buffer :

Binding Buffer	1.5M glycine, 3M NaCl, pH9
Elution Buffer1	0.1M sodium citrate buffer pH5.5
Elution Buffer2	0.2M citrate buffer pH2.5 (or 0.1M Citric Acid pH3.0)
Neutralizing Buffer	1.0 M Tris-HCl, pH 9.0

Delipidation protocol :

Some sera or ascite must be delipidated prior purification

- Add 0.04 ml 10% dextran sulphate solution and 1 M CaCl per ml sample
- Mix for 15min
- Centrifuge at 10 000 g for 10 min
- Discard the precipitate
- Exchange buffer into TBS by dialysis, ultrafiltration or gel filtration. Avoid the use of phosphate buffers.

Sanitization protocol :

This procedure is a guideline only and it is recommended that a protocol be optimized for each specific process.

- Flow 0.1-0.2M NaOH over column for 30 minutes (approx 30-100 cm/hr).
- Immediately equilibrate resin to ~pH7.5 with 5-7 times the column volume of isotonic buffer that has been sterile filtered (0.2µm). 1xPBS or 10xPBS is most common, but other isotonic buffers are acceptable.
- Prior to storing resin, rinse with 5-7 times the column volume with sterile water to remove all NaOH and buffer salts.
- Store column in 20% Ethanol or 0.02% Sodium Azide at 4°C.
- Prior to re-using resin, run 5-7 times the cv of sterile filtered buffer through resin to remove residual storage solution.

Other information

Contact Uptima for any question