

## Affinity support for the purification of IgG antibodies

**Protein G-Affarose****Product Description****Affinity support for the purification of IgG antibodies**

|                          |  |             |
|--------------------------|--|-------------|
| <b>Name:</b>             | Recombinant Protein G immobilized onto a modified agarose gel  |             |
| <b>Part Number:</b>      | UP75196A   | 2ml of gel  |
|                          | UP75196B   | 5ml of gel  |
|                          | UP75196C   | 10ml of gel |
| <b>Matrix:</b>           | highly cross-linked 4% beaded agarose                          |             |
| <b>Binding capacity:</b> | Approx. 18 mg of human IgG per ml of wet gel                   |             |
| <b>Conditionning:</b>    | Protein G Agarose is supplied in 50% of 20% ethanol/PBS        |             |
| <b>Storage:</b>          | +4°C (L). Stable for a minimum of 1 year from date of receipt. |             |

**Technical Information**

Uptima offers this proteinG-agarose reagent 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 G](#) is bacterial protein (Streptococcus group G) that has a high affinity for immunoglobulines G isotypes (IgG) from several species (wider variety and greater affinity than Protein A). It will bind the Fc portion of IgGs from many species including all human and mouse subclasses, and Rabbit, Sheep, Bovine, Goat, Monkey IgGs, Rat IgGs but IgG2b), and Horse IgGT. It does not recognize Human and Mouse IgM, IgA, IgE or serum albumin
- Uptima recombinant protein G is produced in E.coli and purified for achieving best batch to batch consistancy, and better selectivity for IgG. While maintaining the IgG binding site, other binding sites (for albumin and cell surface) have effectively be removed (). ProteinG should not be exposed long time to low pHs, being susceptible to hydrolysis.
- Affarose is a highly cross-linked 4% beaded agarose suitable for affinity purification thanks its hydrophilicity and flow characteristics.
- rProteinG is crosslinked to affarose. The capacity of binding is >18mg of human IgG per mel 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.

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

**Related documents and products**[FT-UP49981](#)

ProteinA-agarose

[Protein A](#)[FT-UP52746](#)

ProteinL-agarose

[Protein L](#)[Protein G](#)

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](mailto: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, NaCl 150-500mM, phosphate 20mM, SDS, Nonidet40 0.1-1%, DOC at 0.1-1%, and protease inhibitors may have been used for cell lysis or solubilization steps. Detergents notably should be removed prior to the proteinG interaction step, i.e. by gelfiltration.

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

In batch procedure, antibody binding is slightly more favorable, but gel washing are 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 require a column with 20µm frits. Circulation by gravity is not recommended because of to low flow rate: use a peristaltic pump or a FPLC system with suitable tubes and fittings (ask Uptima).

### Recommended Column Volumes:

| Biological Fluids        | Recommended bed volume per ml sample |
|--------------------------|--------------------------------------|
| Immune Serum             | 2 ml                                 |
| Supernatant (+10% FCS)   | 200 µl                               |
| Supernatant (serum-free) | 10 µl                                |
| Ascites Fluid            | 2 ml                                 |

### Protocole for Serum purification

- Incubate the sample with 0.45µm filtrated serum, 4hours under constant agitation (batch) or circulation (column) at room temperature  
Longer incubation time may be recommended for achieving higher yield, but lower affinity antibodies could be purified. For sensitive antibodies, incubation may be performed overnight at +4°C
- Wash the gel with PBS (NaCl 150-500mM, phosphate 20mM, PH7.5, # UP307157). Monitor complete removal of unbound molecules (DO280nm <0.05).
- Elute bound antibodies from the gel with Citric or Acetic acid 0.1M pH3 under constant circulation.  
Eluted fractions should be neutralized rapidly (for example with Tris 1M pH9.0) to prevent the degradation of the purified antibodies.
- 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.  
The protein G affarose is stored 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](mailto:uptima@interchim.com) Hotline : 33(0)4 70 03 76 06