Protein G Agarose

Item No	Size
223-51-00	10 ml

Reorder as Catalog No. 223-51-01

Kirkegaard & Perry Laboratories

KPL

INTRODUCTION

Protein G Agarose consists of recombinant protein G, which is produced in *E. coli* and, after purification, is covalently immobilized onto 4% cross-linked agarose beads. Protein G agarose is suitable for the isolation of IgG antibodies using column or immunoprecipitation methods. DNA sequencing of native protein G (from Streptococcal group G) has revealed two IgG-binding domains as well as sites for albumin and cell surface binding (1-6). Protein G has been designed to eliminate the albumin and cell surface binding domains to reduce nonspecific binding while maintaining efficient binding of the Fc region of IgGs. With the removal of these binding domains, Protein G can be used to separate albumin from crude human IgG samples (7).

Covalently coupled Protein G Agarose has been widely used for the isolation of a wide variety of immunoglobulin molecules from several mammalian species. Protein G has greater affinity for many more mammalian IgGs than Protein A (Table 1).

FORM/STORAGE

Protein G Agarose is supplied in a total volume of 15 ml consisting of 10 ml Protein G agarose suspended in 20% ethanol / PBS. Store at 2-8°C. Stable for a minimum of 1 year from date of receipt when stored at 2-8°C. Non-sterile.

SPECIFICATIONS

Ligand density:	~2 mg Protein G/ml drained gel
Bead structure:	4% highly cross-linked agarose
Bead size range:	45 - 165 μm
Recommended working pH:	3 - 9
Binding capacity:	≥18mg/ml Human IgG

Note: Different immunoglobulins derived from the same species and from the same subclass can demonstrate deviations in the binding capacity; Protein G may hydrolyze at low pH.

Table 1. **Relative Affinity of Immobilized Protein G and Protein A for Various Antibody Species and Subclasses of polyclonal and monoclonal IgG's** (8).

Species/ Subclass	Protein G	Protein A
MONOCLONAL		
Human		
IgG_1	++++	++++
IgG_2	++++	++++
IgG_3	++++	---
IgG_4	++++	++++
Mouse		
IgG_1	++++	+
IgG_{2a}	++++	++++
IgG_{2b}	+++	+++
IgG_3	+++	++
Rat		
IgG_1	+	---
IgG_{2a}	++++	---
IgG_{2b}	++	---
IgG_{2c}	++	+
POLYCLONAL		
Rabbit	+++	++++
Cow	++++	++
Horse	++++	++
Goat	++	---
Guinea pig	++	++++
Sheep	++	+/-
Pig	+++	+++
Rat	++	+/-
Mouse	++	++
Chicken	+	---
Human IgG	++++	++++
Human IgM	+	---
Human IgE	+	---
Human IgA	+	---

--- (weak or no binding) → ++++ (Strong binding)

PROCEDURE
PURIFICATION OF IgG MOLECULES

1. **User Supplied Materials**
   a. Buffers: see number 2 below.
   b. Disposable column with frits and reusable caps. KPL recommends using Pharmacia Biotech PD-10 empty disposable columns (Cat. No. 17-0438-01) or equivalent.

2. **Buffer Preparation**
   a. **Wash/Binding Buffer:**
   KPL Cat. No. 50-70-01 or prepare 0.1 M Sodium phosphate, 0.15 M NaCl, pH 7.4.
   b. **Elution Buffer:**
   KPL Cat. No. 50-72-01 or prepare 0.2 M Glycine, pH 2.85.
   c. **Storage Buffer:**
   KPL Buffer Kit, Cat. No. 50-69-01 or prepare 0.01 M NaH_2PO_4, 0.15M NaCl, 2.7 mM KCl, pH 7.4, 20% ethanol.

3. **Sample Preparation:** To insure proper ionic strength and pH are maintained for optimal binding, it is necessary to dilute serum samples, ascites fluid or tissue culture supernatant at least 1:1 with binding buffer. Alternatively, the sample may be dialyzed overnight against wash/binding buffer. KPL recommends using a 12,000 MW cutoff dialysis tubing with at least two buffer exchanges. Remove any particulate matter from the sample by centrifugation or filtration through a 0.8 µm filter.

4. **Column and Resin Preparation:**
   a. Pour 20% Ethanol in the bottom of a petri dish or in a flat bottom container. Float the frit on top of the ethanol. Using the large round end of a 1 ml pipet tip, press the frit firmly into the ethanol to force air out. Repeat this step until the frit is completely wet.
   b. Push the frit into the barrel of the column until it rests firmly on the bottom.
   c. With the cap removed, clip the end of the column to create a hole to allow liquid to flow through.
   d. Wash the frit with 5 column volumes of 1X Wash/Binding Buffer.
   e. Prepare a 1:1 suspension of resin in 1X Wash/Binding buffer. The required amount of agarose per mg immunoglobulin to be purified can be estimated by the binding capacity.

Recommended Column Volumes

Antibody Source	Recommended bed volume (ml) per ml sample
Immune Serum	2 ml
Tissue Culture Supernatant (with 10% fetal bovine serum)	0.2 ml
Tissue Culture Supernatant (serum-free)	0.01 ml
Ascites Fluid	2 ml

   f. Pour slurry into column. Allow column to flow by gravity to pack the column bed.
   g. Equilibrate the packed affinity resin with 10 column volumes (CV) of the wash/binding buffer. For example, if the packed bed is 1 ml, equilibrate with 10 ml Wash/Binding Buffer.

5. **Sample Purification**
   a. Gently apply sample to the column by layering onto the top of the resin. Be careful not to disturb the bed surface.
   b. Wash column with 10 CV of the 1X Wash/Binding buffer, or until the absorbance of eluate at 280 nm approaches the background level.
   c. Before beginning the elution step, set up enough tubes to collect the entire elution volume as 1 ml fractions (4 CV will be used to elute the antibody). To each collection tube add 240 µl 5X Wash/Binding Buffer. To elute the antibody, gently add 1 ml 1X Elution Buffer to the top of the resin collecting the eluate in a prepared collection tube. Repeat until the entire volume has been collected up to 4 column volumes. **Note:** If the eluate is to be collected in a single bulk volume, add 240 µl 5X Wash/Binding Buffer per ml Elution Buffer to the collection vessel before starting the elution. Elution of bound immunoglobulin can be monitored by absorbance at 280 nm, if desired.

5. **Column Regeneration:** Once the sample has been eluted, wash the affinity matrix with 2 CV of elution buffer. Re-equilibrate the column with at least 10 CV of 1X Wash/Binding Buffer. When column is equilibrated, pH of eluate will be the same as that of the wash/binding buffer.

6. **Clean-in-Place:** With certain applications, substances which contain denatured proteins or lipids do not elute in the regeneration procedure. The following steps can be taken to clean the column:
   a. To remove strongly bound hydrophobic proteins, lipoproteins and lipids, wash the column with a non-ionic detergent (e.g. 0.1% Triton X-100) at 37°C, with a contact time of ~1 minute.
   b. Immediately re-equilibrate the column with 5 - 10 CV of 1X Wash/Binding Buffer.
   c. As an alternative, wash the column with 70% ethanol. Allow the column to stand for 12 hours. Re-equilibrate the column with 5 - 10 CV of 1X Wash/Binding Buffer.
   d. To remove precipitated or denatured substances, wash the column with 2 CV of 6M guanidine hydrochloride. Immediately re-equilibrate the column with 5 - 10 CV of 1X Wash/Binding Buffer (see step 5).

6. **Resin Storage:** Store affinity matrix in Storage Buffer at 2-8°C. **Do not** store the matrix frozen or at room temperature. The matrix can be stored in the column by

sealing the outlets or removed from the column and stored as a slurry.

IMMUNOPRECIPITATION

For immunoprecipitation protocols, see references 9-12.

PRODUCT SAFETY AND HANDLING

This product is considered hazardous as defined by The Hazard Communication Standard (29 CFR 1910.1200). Avoid contact with skin and eyes. Consult the Material Safety Data Sheet for precautions and disposal.

REFERENCES

1. Guss, B., et.al. (1986). *EMBO J.,* 5: 1567 – 1575.
2. Akerstrom, B., Nielsen, E. and Bjorck, L. (1987). *J. Biol. Chem.,* 262: 13388 - 13391.
3. Sjobring, U., et. al. (1988). *J. Immunol.,* 140: 1595 - 1599.
4. Fahnestock, S. (1987). *TIBS,* 79 - 83.
5. Olsson, A., et. al. (1987). *Eur. J. Biochem*. 168: 391 - 324.
6. Fahnestock, S., et. al. (1986). *J. Bacteriol*., 176: 870 - 880.
7. Goward, C.R., et. al. (1990). *Biochem. J*., 267: 171-177.
8. Harlow, E. and Lane, D. eds.(1988). Antibodies, A Laboratory Manual. Cold Spring Harbor Laboratory, N.Y., 617-618.
9. Thurston, C.F. and Henley, L.F. (1988). *In* Walker, J.M., ed. Methods in Molecular Biology, Vol. 3-New Protein Techniques. Humana Press: Clifton, NJ, 149-158.
10. Kent,U.M. (1994). *In*: Javois, L.C., ed. Methods in Molecular Biology, Vol. 34-Immunocytochemical Methods and Protocols. Humana Press: Totowa, NJ, 37-41.
11. Bollag, D.M., Rozycki, M.D. and Edelstein, S.J. (1996). Protein Methods, 2nd ed. Wiley-Liss: New York, 87-90.
12. Goward, C.R. et. Al., *Biochem.J.* (1990), 267, 171-177.

RELATED PRODUCTS

Protein G Agarose Kit	Cat. No. 553-51-00
Protein A Agarose Kit	Cat. No. 553-50-00
Protein A Agarose	Cat. No. 223-50-01
Wash/Binding Buffer	Cat. No. 50-70-02
Elution Buffer	Cat. No. 50-68-02

See KPL's catalog for a complete list of antibodies, substrates, Western blotting, and ELISA kits