SEPHAROSE™ PROTEIN G - PG50-00-0002

Rockland's Sepharose Protein G is prepared by covalently coupling recombinant Protein G to 6% crosslinked sepharose beads. The coupling technique is optimized to give a high binding capacity for IgG, resulting in IgG binding greater than 20 mg of human or rabbit IgG per cc of settled beads.

I. PRODUCT CONTENTS

Contents	Catalog Number	Size
SEPHAROSE™ PROTEIN G	PG50-00-0002	2 mL

II. PRODUCT CHARACTERISTICS

Specificity	Genetically engineered Ig-binding protein
Stability	pH range of 2 to 10
Binding Capacity	≥ 20 mg human or rabbit IgG/mg of beads
Flow	High rate; 0.85cm/min
Composition	20% Ethanol/Water. Thimerosal Free
50% slurry	1cc settled bead per 2mL suspension

III. STORAGE CONDITIONS

The Protein G Sepharose should be stored in the refrigerator (2-8°C). Do not freeze. Do not let the resin bed dry.

IV. APPLICATIONS

Immunoprecipitation at 20 µL per immunoprecipitation reaction. Pull down antibody:antigen complexes in immunoprecipitation experiments. High affinity & specificity to the Fc portion.

IgG purification of monoclonal and polyclonal antibodies from serum, tissue culture, ascites fluid, or supernatants.

A. PURIFICATION EQUIPMENT AND REAGENTS

- Binding Buffer: PBS/TBS/0.15 M sodium chloride in 50 mM sodium borate, pH 8.0
- 0.5M NaCl
- Elution Buffer: 0.1 M citric acid, pH 2.75
- Neutralization Buffer: 1 M Tris-HCl, pH9
- 20 % Ethanol/H2O

V. ANTIBODY PURIFICATION PROTOCOL

- Carefully pack the beads into resin avoiding air bubbles.
- Equilibrate the column with 5 resin bed volumes of Binding Buffer and allow the buffer to drain through the column. Do not let the resin bed dry.
- Dilute serum sample 1:1 with Binding Buffer and mix well without causing bubbles in the solution.
- Apply the diluted serum onto the resin for binding. Do not let the resin bed dry.
- Collect the flow-through.
- Reapply the flow-through to the column and collect new flow-though. Repeat depending on size of resin and serum sample.
- Wash column 4-5 times with 5 resin bed volumes of Binding Buffer containing 0.5M NaCl.
- Wash column 4-5 times with 5 resin bed volumes of Binding Buffer.
- Elute the antibodies 1 time with 3-5 resin bed volumes of Elution Buffer.
  - Collect fractions in numbered micro centrifuge tubes containing Neutralization Buffer.
  - 100µL Neutralization Buffer per mL Elution buffer
- Protein Concentration:
  - Measure UV absorbance at 280nm and combine fractions with highest absorbance.
  - 1 OD280=0.73mg/mL IgG
- Regenerate resin:
  - 5 resin bed volumes of Elution Buffer.
  - 5 resin bed volumes of distilled water.
- Store resin in 20% ethanol/H20 at 2-8°C.

- **Note: Columns may be regenerated 8-10 times without significant loss of binding capacity.**

VI. IMMUNOPRECIPITATION PROTOCOL

Immunoprecipitation (IP) is performed by either adding the selected primary antibody to Protein G beads followed by adding the sample antigen, or by first forming an antigen-antibody complex and then incubating the complex with Protein G beads. Bound antigen-antibody complex is then eluted using different elution methods.

IMMUNOPRECIPITATION EQUIPMENT AND REAGENTS

- Lysis Buffer (with protease inhibitors)
- Cold PBS
- SDS-PAGE sample buffer with reducing agent
- Protease inhibitors
- Blocking Buffer
- TBST-T

A. General guidelines

- The amount of antibody captured depends on the concentration of antibody and Protein G beads in the starting sample.
- For standard immunoprecipitation use PBS or TBS for antibody binding and washing steps. However, these may be substituted by other buffers of choice, such as phosphate buffers, lysis buffer (e.g. RIPA, NP40), or HEPES. The recommended elution buffer may also be substituted by alternative low pH-, high pH- or high salt buffers, depending on the target protein and downstream application.

- Immunoprecipitation

1. Add 1-10µg of immunoprecipitation antibody to the tube containing the cold precleared cell lysate.

Note: This concentration of monoclonal antibody is suggested as a starting point. Using as little IP antibody as possible minimizes potential contamination of SDS reduced samples with non-reduced immunoprecipitating antibody light chain. It is not recommended to use more than 10µg (per ml) or 5µg per lane.

2. Incubate at 4°C for 1 hour on a rocking platform or orbital shaker.

3. Add at least 50µL of pre-equilibrated bead slurry to capture the immune complexes.

4. Incubate for 1 hour or overnight at 4°C on a rocking platform or orbital shaker.

Note: Step 1 and 3 can be combined into a single incubation step.

5. Centrifuge the tube at 2,500xg for 30 seconds at 4°C.

6. Carefully remove supernatant completely and wash the beads 3-5 times with 500µL of cold Lysis Buffer, centrifuge to pellet beads in between washes. To minimize background, care should be given to remove the supernatant completely following each wash.

7. After the last wash, carefully aspirate supernatant and add 50µL of 1X Laemmli sample buffer (or any equivalent SDS-PAGE sample loading buffer) to bead pellet.

Note: It is critical to add reducing agent. Prior to use, prepare 2X SDS Reducing Sample Buffer by adding 1M DTT to 2X SDS Sample Buffer resulting in a final concentration of 50 mM DTT. NuPAGE or standard Laemmli buffer may also be used with the addition of reducing agent (50 mM DTT or 2% β-mercaptoethanol, final).

8. Vortex and heat to 90-100°C for 10 minutes.

9. Centrifuge at 10,000xg for 5 minutes, carefully collect supernatant and load onto the gel.

10. Alternatively, the supernatant samples can be collected, transferred to a clean tube and frozen at -80°C if the gel is to be run at a later stage.

11. Follow manufacturer's instructions for SDS-PAGE.

VII. RELATED PRODUCTS

Contents	Catalog Number	Size
TRUEBLOT PROTEIN G Magnetic Beads	PG00-18-2	2 mL
TRUEBLOT PROTEIN A Magnetic Beads	PA00-18-2	2 mL
SEPHAROSE™ PROTEIN A	PA50-00-0002	2 mL
SEPHAROSE™ PROTEIN A	PA50-00-0005	5 mL

VIII. RELATED BINDING

Polyclonal IgG	Protein A	Protein G	Protein A/G
Bovine	W	S	S
Bovine IgG1	nb	S	S
Bovine IgG2	S	S	S
Cat	S	W	S
Chicken	nb	nb	nb
Dog	S	W	S
Dog IgA (some)	S	W	--
Dog IgM (some)	S	W	--
Donkey	M	S	S
Goat	W	S	S
Goat IgG1	nb	W	S
Goat IgG2	S	W	S
Guinea Pig	S	W	S
Guinea Pig IgG1	S	W	--
Guinea Pig IgG2	S	W	--
Hamster	W	W	--
Horse	W	S	S
Horse IgG (ab)	W	nb	W
Horse IgG (c)	W	nb	W
Horse IgG (T)	nb	S	S
Human	S	S	S
Human IgA	W	nb	W
Human IgA1	W	M	M
Human IgA2	S	nb	--
Human IgD	nb	nb	W
Human IgE	M	nb	M
Human IgG1	S	S	S
Human IgG2	S	S	S
Human IgG3	nb	S	S
Human IgG4	S	S	S
Human IgM (some)	W	nb	W
Monkey (Rhesus)	S	S	S
Mouse	S	S	S
Mouse IgG1	W	M	M
Mouse IgG2a	S	S	S
Mouse IgG2b	S	S	S
Mouse IgG3	W	S	S
Mouse IgM	nb	nb	nb
Rabbit	S	S	S
Rat	W	M	M
Rat IgG1	W	M	M
Rat IgG2a	nb	S	S

Rat IgG2b	nb	W	W
Rat IgG2c	W	W/S	M
Sheep	W	S	S
Sheep IgG1	nb	S	S
Sheep IgG2	S	S	S
Swine	S	W	S
Swine IgA (some)	S	W	--
Swine IgM (some)	S	W	--
S = Strong binding			
M = Medium binding			
W = Weak binding			
nb = no binding			
w/s = weak overall, but affinity greater than protein A			
-- = no data			
Binding of immunoglobulins to Protein A and G is pH dependent.			
Compiled from various sources.			