SEPHAROSE™ PROTEIN A/G - PAG50-00-0002

Rockland's Sepharose Protein A/G is a suspension of sepharose beads conjugated to native protein A derived from Staphylococcus aureus and recombinant Protein G. The coupling technique is optimized to give a high binding capacity for IgG, resulting in IgG binding greater than 20 mg of human IgG per cc of settled beads.

I. PRODUCT CONTENTS

Contents	Catalog Number	Size
SEPHAROSE™ PROTEIN A/G	PAG50-00-0002	2 mL

II. PRODUCT CHARACTERISTICS

Specificity	Ig-binding protein
Stability	pH range of 2 to 10
Binding Capacity	≥ 20 mg human IgG/mL
Flow	High rate; 1300cm/hr
Composition	20% Ethanol/Water. Thimerosal Free
Bead Size	approximately 45-165 µm when swollen
25% slurry	0.5cc settled bead per 2mL suspension

III. STORAGE CONDITIONS

The Protein A/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 pH 7.0
- Elution Buffer: 0.1 M citric acid pH 3.0
- Neutralization Buffer: 1 M Tris-HCl pH 9.0

V. ANTIBODY PURIFICATION PROTOCOL

- Carefully pack the beads into resin avoiding air bubbles.
- If the column contains 20% ethanol wash the resin with 5 resin bed volumes of distilled water and allow the buffer to drain through the column. Do not let the resin bed dry.
- Use a linear flow rate of 50-100cm/hr.
- 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.
- Use a linear flow rate of 150cm/hr.
- 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 until the absorbance reaches baseline.
- Elute the antibodies by step or linear gradient method:
    - Step: load 5 resin bed volumes of Elution Buffer.
    - Linear Gradient: create a shallow gradient over 20 column volumes to allow separation of proteins with similar binding strength.
        - Collect fractions in numbered micro centrifuge tubes containing Neutralization Buffer.
        - 100µL Neutralization Buffer per mL Elution buffer
- After elution wash resin with 5-10 resin bed volumes of Binding Buffer for next purification.
- 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 A/G beads followed by adding the sample antigen, or by first forming an antigen-antibody complex and then incubating the complex with Protein A/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 A/G beads in the starting sample.
- For standard immunoprecipitation use PBS 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 G	PG50-00-0002	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.			