

# rProtein G resin

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## Introduction

Protein G Agarose is Protein G from Sino Biological immobilized to 4% agarose. Protein G Agarose is an affinity chromatography medium designed for easy, one-step purification of classes, subclasses and fragments of immunoglobulins or recombinant proteins containing Fc regions from biological fluids or cell culture media.

Protein G is a bacterial cell wall protein isolated from group G *Streptococci*, which binds to the Fc region of IgG from a variety of mammalian species. Native Protein G has IgG binding domains and also sites for albumin and cell-surface binding. The latter have been eliminated from recombinant Protein G to reduce nonspecific binding. Consequently, recombinant Protein G can be used to separate IgG from crude human or mouse serum samples.

For the tertiary structures of Protein A and Protein G are very similar, the potential applications of Protein G include nearly all of the current applications of protein A. However Protein A and Protein G has different binding characteristics because their amino acid compositions differ significantly. Compared to protein A, Protein G has greater affinity ability and binds more strongly for most mammalian IgGs, especially for certain subclasses including human IgG3, mouse IgG1 and rat IgG2a. Unlike Protein A, Protein G does not bind to human IgM, IgD and IgA.

Sino Biological Protein G agarose is made and supported for industrial scale especially the manufacture of healthcare products. For its high physical, chemical stability and high batch-to-batch reproducibility, it is ideal for all stages of unit operations from process development through scale-up and production.

**Table 1. Characteristics of Protein G Agarose**

| | |
|---|---|
| Ligand | Recombinant *Streptococcal* Protein G |
| Dynamic binding capacity | ~18 mg/ml |
| Matrix | Spherical, 4% agarose |
| Average particle size | 90um(45-165um) |
| Maximum linear flow rate | >150 cm/h |
| Maximum Pressure: | 0.3 MPa |
| pH stability Long term: | pH 3-9 |
| Short term: | pH 2-10 |
| Storage | 20% ethanol at 2°C to 8°C |

# Operation

**A: Purification using pump**

1 **Buffer preparation**

Binding Buffer: 50mM Tris-HCl, 100mM NaCl, pH8.0
Elution Buffer: 0.1M glycine, pH 2-3
Neutralization Buffer: 1M Tris-HCl, pH8-9
Clean buffer: 0.1% Triton™ X-100 or 70% ethanol
Storage buffer: 20% ethanol and/or 0.02% sodium azide

**2 Preparing the chromatography medium**

Protein G Agarose is supplied in 20% ethanol. Prepare slurry by replacing the 20% ethanol with Binding Buffer. The Binding Buffer should not contain agents which significantly increase the viscosity. The column may be equilibrated with viscous buffers at reduced flow rate after packing is completed. For batch procedures remove the ethanol by washing the medium on a medium-porosity sintered glass funnel.

**3 Packing Protein G Agarose**

3.1 Equilibrate all material at the chromatography performed temperature.
3.2 De-gas all buffers which will be used.
3.3 Flush the end pieces with Binding Buffer to eliminate air of the column dead spaces. Make sure no air has been trapped under the column net. Close the column outlet with a few centimeters of Binding Buffer remaining in the column.
3.4 Pour the slurry into the column. Pouring the slurry down a glass rod held against the wall of the column will minimize the introduction of air bubbles.
3.5 Immediately fill the remainder of the column with Binding Buffer.
3.6 Insert the adapter at an angle into the column, ensuring that no air is trapped under the net. Connect the column to a pump.
3.7 Slide the plunger slowly down the column so that the air above the net and in the capillary tubings is displaced by buffer.
3.8 Lock the adapter in position, open the column outlet and start the flow of buffer. Pass buffer through the column at the packing flow rate until the medium bed is stable. Re-position the adapter onto the medium surface as necessary.
3.9 The column is now packed and equilibrated and ready for use.

**Note:** Do not exceed 75% of the packing flow rate in subsequent purification procedures.

**4 Purification procedures**

4.1 Equilibrate the packed Protein G Agarose and all buffers to room temperature.
4.2 Equilibrate the column by Binding Buffer.

4.3 Apply the sample. For the best binding capacity, the serum samples, ascitic fluid or cell culture supernatant should be diluted at least 1:1 with Binding Buffer.
4.4 **Note:** Plasma may become hazy upon dilution with the Binding Buffer because of lipoprotein precipitation. Centrifuge the diluted sample at 10,000 × g for 20 minutes and apply the supernatant to the equilibrated Protein G Agarose.
4.5 Wash the Protein G column with 5-10 bed volumes of Binding Buffer.
4.6 Elute antibodies with Elution Buffer. Immediately adjust eluted sample to physiologic pH by adding about 1/20 volume of Neutralization Buffer. Monitor the elution by measuring the absorbance at 280 nm or other protein detection method.
4.7 After elution, the resin should be immediately washed with 3 to 5 bed volumes of elution buffer and then re-equilibrated with Binding Buffer. With this procedure the resin will be regenerated.
4.8 In some applications, substances like denatured proteins or lipids do not elute in the regeneration procedure. These can be removed by cleaning-in-place procedures. Washing the column with a non-ionic detergent (e.g. Triton™ X-100), 0.1%, at 37°C, contact time of one minute or wash the column with 70% ethanol and remain for 12 hours. Re-equilibrate with at least 5 bed volumes of Binding Buffer.
4.9 For storage, keep the medium at 2°C to 8°C in a suitable bacteriostat, e.g. 20% ethanol and/or 0.02% sodium azide.

**Notice:** The resin must not be frozen.

**B: Gravity-flow column procedures for purification**

1 **Buffer preparation:**
Same as **purification using pump**.

**2 Antibody purification**

2.1 Equilibrate the Protein G Agarose and all buffers to room temperature.
2.2 Following the instructions provided with the columns carefully pack the column with resin slurry.
2.3 Equilibrate the column by adding the Binding Buffer and allowing the solution to drain through the column. **Note:** Remove the top cap first.
2.4 Apply the sample. For the best binding capacity, the serum samples, ascitic fluid or cell culture supernatant should been diluted at least 1:1 with Binding Buffer. **Note:** Plasma may become hazy upon dilution with the Binding Buffer because of lipoprotein precipitation. Centrifuge the diluted sample at 10,000 × g for 20 minutes and apply the supernatant to the equilibrated Protein G Agarose.
2.5 Apply the diluted sample to the column and allow it to flow completely into the resin. Do not allow the resin bed to run dry.

2.6 Wash the Protein G column with 5-10 bed volumes of the Binding Buffer. **Note:** Collect the flow-through into 2ml fractions. The last fractions should have absorbance similar to Binding Buffer alone.
2.7 Elute antibodies with Elution Buffer and collect 0.5-1 ml fractions. Immediately adjust eluted fractions to physiologic pH by adding the Neutralization Buffer. Monitor the elution by measuring the absorbance at 280 nm or by protein assay.
2.8 Pool the eluted IgG fractions that contain the highest absorbance. The purified antibodies can be used directly for SDS-PAGE, or buffer exchanged by dialysis or desalting column for the specific downstream application.
2.9 Regenerate column by washing with 3-5 bed volumes of Elution Buffer and then re-equilibration with Binding Buffer.
2.10 For storage, keep the medium at 2°C to 8°C in a suitable bacteriostat, e.g. 20% ethanol and/or 0.02% sodium azide. Store columns upright at 4°C.

**Notice:** The resin must not be frozen.

**C: Immunoprecipitation (IP) procedures using Protein G Agarose**

**Additional materials required**

1.5-2 ml micro centrifuge tube
IP Buffer: 25mM Tris, 150mM NaCl, pH 7.2
Elution Buffer: 0.1 M Glycine, pH 2-3
Electrophoresis Loading Buffer: Lane marker reducing sample buffer (4X)
Neutralization Buffer: 1 M Tris-HCl pH8-9
Antigen Sample: Antigen-containing lysate or sample prepared in IP Buffer or other buffer that is compatible with both the desired antibody binding interaction and the binding of antibody to Protein G

**Immunoprecipitation procedures**

1.1 Combine 50-1,000 µl of the Antigen Sample and optimized amount of antibody in a micro centrifuge tube. Incubate overnight at 4°C.
1.2 Add 100 µl of Protein G Agarose slurry to the antigen-antibody complex. Incubate with gentle mixing for 4 hours at 4°C. **Note:** Always keep samples on ice.
1.3 Add 0.5 ml of IP Buffer, centrifuge for 2 minutes at 3,000 × g and discard supernatant. Repeat this step for several times.
1.4 Add 50 µl of Elution Buffer and incubate for 5 minutes to elute the immune complex. Centrifuge for 2 minutes at 3,000 × g and collect the supernatant. Repeat this step and combine the two supernatant fractions.
1.5 Adjust eluate to physiological pH by adding Neutralization Buffer. The IP products can be used directly for SDS-PAGE, or buffer exchanged by dialysis or desalting column for the specific downstream application.

## Troubleshooting

| Problem | Possible Cause | Solution |
|---|---|---|
| The flow rate of the column is very low (<0.5 ml/minute). | Tiny air bubbles from buffer or particles from sample block the column or the gel pores. | De-gas buffers and filtrate samples. Do not allow the column to dry. |
| A considerable amount of sample has been loaded, but no specific antibody of interest is detected. | The concentration of antibody of interest is very low. | Purify the antibody using the specific antigen coupled resin |
| The antibody is degraded. | The antibody is sensitive to low-pH elution buffer | Neutralize the eluted fractions immediately after elution. |
| No antibody is detected in any elution fraction. | The IgG subclass does not bind to Protein G. | Try other affinity chromatography media to purify the antibody, such as Protein A or Protein L Resin. |

## Related products

| Product | Package | Cat. No. |
|---|---|---|
| rProtein G | 1mg, 5mg, 10mg | 13103-PNAE |
| rProtein A | 10mg, 1g, 10g | 10600-P07E |
| rProtein A resin | 5ml, 25ml | 10600-P07E-RN |
| rProtein L | 1mg, 5mg, 10mg | 11044-H07E |
| rProtein L resin | 5ml, 25ml | 11044-H07E-RN |