Protein A and Protein G Spin Plates for IgG Screening

Introduction

The Protein A and Protein G Spin Plates for IgG Screening offer a high-throughput format for quick purification and screening of antibodies and for immunoprecipitation (IP). Each well of the 96-well spin plate can process 10-100 µl of serum, cell culture supernatant or ascites fluid samples. The Protein A and Protein G resin purifies by binding IgGs, primarily through their Fc region, in a buffer that facilitates the interaction. Non-relevant proteins, such as albumin and transferrin, are removed by washing and the purified antibody is then recovered using low-pH elution conditions.

Although Protein A and Protein G are similar proteins, their amino acid compositions differ significantly, resulting in different binding characteristics (Table 1). Protein A interacts with IgG from several species, including human and rabbit, and selectively interacts with some subclasses. For example, human IgG1, IgG2, and IgG3 bind strongly but IgG3 does not. Also the majority of rat IgGs and mouse IgG1 do not bind to Protein A. For purifying mammalian IgGs that do not bind well to Protein A, Protein G is useful because it binds with significantly greater capacity than Protein A to several IgG subclasses such as human IgG3, mouse IgG1, and rat IgG2a. Protein G, however, does not bind to human IgM, IgD and IgA. For a more extensive listing of binding characteristics for Protein A and Protein G, please see the Technical Resources – Tech Tips section of our website.

Table 1. Purification characteristics of IgG species using Protein A and Protein G.

<table>
<thead>
<tr>
<th>Source</th>
<th>Protein A</th>
<th>Protein G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>G</td>
<td>G</td>
</tr>
<tr>
<td>Mouse</td>
<td>G</td>
<td>G</td>
</tr>
<tr>
<td>Rabbit</td>
<td>G</td>
<td>G</td>
</tr>
<tr>
<td>Rat</td>
<td>W</td>
<td>M</td>
</tr>
<tr>
<td>Goat</td>
<td>W</td>
<td>G</td>
</tr>
<tr>
<td>Cow</td>
<td>W</td>
<td>G</td>
</tr>
<tr>
<td>Sheep</td>
<td>W</td>
<td>G</td>
</tr>
<tr>
<td>Horse</td>
<td>W</td>
<td>G</td>
</tr>
<tr>
<td>Guinea Pig</td>
<td>G</td>
<td>W</td>
</tr>
<tr>
<td>Pig</td>
<td>G</td>
<td>W</td>
</tr>
<tr>
<td>Chicken</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Hamster</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>Donkey</td>
<td>M</td>
<td>G</td>
</tr>
</tbody>
</table>

Legend: G = good purification; M = medium purification; W = weak purification; N = does not purify
Important Product Information

- The spin plates are compatible with centrifugation and positive pressure systems.
- The Protein A and Protein G Spin Plates must be balanced throughout the procedure with a balance plate (Product No. 45205) filled with an appropriate volume of water.
- Each well contains 50 µl of resin, which can purify 10-100 µl of serum, cell culture supernatant, or ascites fluid. Be aware that antibodies from fetal bovine serum (FBS) culture supplement will co-purify with the antibody of interest.
- For optimal recovery, use a sample size such that the expected IgG load is less than 80% of the maximum binding capacity (see page 1 for binding capacities). The total IgG content of serum is approximately 10-15 mg/ml. The concentration of antibody in tissue culture supernatant and ascites fluid vary considerably among hybridoma clones.
- Using buffer formulations other than those indicated could significantly alter the binding capacity and the wash volumes required for efficient purification.

Additional Materials Required

- Plate or orbital shaker
- Variable-speed centrifuge with rotor and carriers capable of handling stacked plates (4.4 cm height) at 1,000 × g
- Balance Plate (Product No. 45205)
- Binding Buffer: BupH™ PBS Buffer (Product No. 28372) or 0.1 M sodium phosphate, 0.15 M sodium chloride, pH 7.2
- Elution Buffer: IgG Elution Buffer (Product No. 21004) or 0.1 M glycine, pH 2-3
- Neutralization Buffer: 20 ml of high-ionic strength alkaline buffer such as 1 M phosphate or 1 M Tris (pH 7.5-9)
- Optional: Zeba™ 96-well Desalt Spin Plates (Product No. 89807) for buffer exchange
- Optional: polypropylene collection plates (Product No. 89813) for collecting and storing extra fractions (i.e., flow-through fraction or samples)
- Optional: Sealing Tape for 96-well plates (Product No. 15036)

Procedure for Purification/Screening of IgG

1. Equilibrate the spin plate and all buffers to room temperature (~30 minutes).
2. Remove the bottom seal from the plate and place the plate on top of the wash plate. Remove the top seal and equilibrate the plate by adding 200 µl of Binding Buffer to each well.
3. Place the plate assembly into a centrifuge with a 96-well plate-carrier and centrifuge for 1 minute at 1,000 × g. Discard the flow-through. Repeat this step once.
4. Replace the purification plate on top of the wash/collection plate.
5. Dilute samples at least 1:1 with Binding Buffer before adding to the plate to maintain proper ionic strength and pH for optimal binding.
6. Apply the diluted samples (total volume is 20-200 µl) to the wells. To expel the entire sample, carefully touch the pipette tips to the resin.
7. Place the plate assembly on a plate or orbital shaker and incubate for 30 minutes with moderate agitation.
8. Centrifuge the plate assembly at 1,000 × g for 1 minute. Discard the flow-through or use extra collection plates (Product No. 89813) to collect and store the flow-through.
   **Note:** If the sample contains more IgG than can bind (or an antibody type that does not bind), the flow-through will contain excess antibody. By saving the flow-through, non-bound antibody can be recovered and evaluated.
   **Note:** More antibody might bind by reapplying the flow-through to the corresponding wells of the purification plate.
9. Place the purification plates on the wash/collection plate. Wash the resin by adding 500 µl of Binding Buffer to each well and then centrifuging at 1,000 × g for 1 minute. Repeat this step three more times, discarding the flow-through each time.
10. Discard the wash/collection plate; alternatively, rinse it once with 70% ethanol, rinse three times with ultrapure water, and dry and save it for future use.

11. Add 20 µl of Neutralization Buffer to each well of the wash/collection plate.

12. Place the purification plate on the wash/collection plate. Add 200-400 µl of Elution Buffer and incubate with agitation for 1 minute. Centrifuge at 1,000 × g for 1 minute to collect the elution. Repeat this step two more times.

   **Note:** In most applications, the first two fractions will contain the majority of purified sample.

13. Discard the plates, or regenerate the resin by washing three times with 400 µl of Elution Buffer and three washes with 400 µl of 0.02% sodium azide in ultrapure water.

14. For storage, seal the bottom of the plate, add 100 µl of 0.02% sodium azide in ultrapure water or Binding Buffer and then seal the top of the plate. Place the plate in the original sealable bag and store at 4ºC.

**Procedure for Immunoprecipitation (IP)**

1. Prepare the antigen-antibody complex by combining the antigen-containing sample prepared in Binding Buffer with the optimized amount of antibody and incubate overnight at 4ºC.

   **Note:** Use any Binding Buffer that is compatible with both the desired antibody interaction and the antibody binding to Protein A or Protein G.

2. Equilibrate the spin plate and all buffers to room temperature (~30 minutes).

3. Remove the bottom seal from the plates and place one plate on top of a wash/collection plate. Remove the top seal and equilibrate the plate by adding 200 µl of Binding Buffer to each well.

4. Place the plate assembly into a centrifuge with a 96-well plate-carrier and centrifuge for 1 minute at 1,000 × g. Discard the flow-through. Repeat this step once.

5. Place the purification plates on top of the wash/collection plate.

6. Apply 20-200 µl of the antibody-antigen sample to each well. To expel the entire sample, carefully touch the pipette tips to the resin.

7. Place the plate assembly on a plate or orbital shaker and incubate for 1 hour with moderate agitation.

8. Centrifuge the plate assembly at 1,000 × g for 1 minute. Discard the flow-through or use extra collection plates (Product No.89813) to collect and store the flow-through.

   **Note:** If the sample exceeds the column’s binding capacity, the flow-through will contain the excess, which can be recovered. Also, more antibody-antigen sample might bind by reapplying the flow-through to the corresponding wells of the purification plate and repeating steps 7-8.

9. Place the purification plate on the wash plate, and wash resin by adding 400 µl/well of Binding Buffer and then centrifuging at 1,000 × g for 1 minute. Repeat this step three more times, discarding the flow-through each time.

10. Discard the wash/collection plate; alternatively, rinse it once with 70% ethanol, rinse three times with ultrapure water, and dry and save it for future use.

11. Add 20 µl/well of Neutralization Buffer to the wash/collection plate.

12. Place the purification plate on the collection plate. Add 200-400 µl of Elution Buffer and incubate with agitation for 1 minute. Centrifuge the plate assembly at 1,000 × g for 1 minute to collect the eluate. Repeat this step two more times.

13. Discard the plates, or regenerate resin by washing three times with 400 µl of Elution Buffer and three times with 400 µl of 0.02% sodium azide in ultrapure water.

14. For storage, seal the bottom of the plate, add 100 µl of 0.02% sodium azide in ultrapure water, and then seal the top of the plate. Place the plate in the original sealable bag and store at 4ºC.
## Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Considerable antibody purified, but no antibody of interest detected</td>
<td>Antibody of interest is at low concentration</td>
<td>Affinity-purify the antibody using the specific antigen coupled to an activated support such as AminoLink® Plus Immobilization Kit (Product No. 44894)</td>
</tr>
<tr>
<td>Antibody of interest purified, but it is degraded (determined by lack of function in downstream assay)</td>
<td>Antibody is sensitive to low-pH elution buffer</td>
<td>Try Gentle Ag/Ab Binding and Elution Buffer (See Related Products)</td>
</tr>
<tr>
<td></td>
<td>Downstream application is sensitive to neutralized Elution Buffer</td>
<td>Desalt the eluted sample into a suitable buffer</td>
</tr>
<tr>
<td>No protein detected in any elution fraction</td>
<td>Sample devoid of antibody species or class that binds to Protein A or Protein G</td>
<td>Refer to Binding Characteristics Table for Protein A and Protein G (See Additional Information Section)</td>
</tr>
</tbody>
</table>

### Additional Information

Please visit our website for additional information on this product including the following:

- Tech Tip: Protein Stability and Storage

### Related Products

- **45208** Melon™ Gel Spin Plate Kit for IgG Screening
- **45206** Melon Gel IgG Spin Purification Kit, sufficient reagents to purify up to 3 ml of serum
- **28372** BupH™ Phosphate Buffered Saline Pack, 40 packs
- **21004** IgG Elution Buffer, 1 L
- **21027** Gentle Ag/Ab Elution Buffer, 500 ml
- **89806** Protein Stabilizing Cocktail (4X), 10 ml
- **45216** Saturated Ammonium Sulfate, 1 L
- **89807** Zeba 96-well Desalt Spin Plates, 2 pack

### References


This product (“Product”) is warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts (“Documentation”) and to be free from defects in material and workmanship. Unless otherwise expressly authorized in writing, Products are supplied for research use only. No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than the original purchaser of the Product (“Buyer”).

No other warranties, express or implied, are granted, including without limitation, implied warranties of merchantability, fitness for any particular purpose, or non infringement. Buyer’s exclusive remedy for non-conforming Products during the warranty period is limited to replacement of or refund for the non-conforming Product(s).

There is no obligation to replace Products as the result of (i) accident, disaster or event of force majeure, (ii) misuse, fault or negligence of or by Buyer, (iii) use of the Products in a manner for which they were not designed, or (iv) improper storage and handling of the Products.

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