# Pearl™ IgG Purification Resin Spin Plates

## 96-Well Spin Format For the Purification of Immunoglobulin G from Serum

## (Cat. # 786-995)

## INTRODUCTION

The Pearl™ IgG Purification Spin Plate kit allows for the one-step purification and screening of immunoglobulin G from serum. The plates enable the purification of 96 samples of 20-100µl in <15 minutes. The Pearl resin binds the high abundant, non-IgG proteins (i.e. albumin) and allows the IgG molecules to pass through in a physiological buffer. The IgG molecules can be stored or used in downstream applications without further clean-up, such as ammonium sulfate precipitation.

The Pearl™ IgG Purification Spin Plates can purify ~1mg human IgG/well.

## KIT COMPONENTS

| Description | Size |
|---|---|
| Pearl™ IgG Purification Spin Plates (100µl resin/well) | 2 plates |
| IgG Isolation Buffer [100X] | For 1L |
| Wash/ Collection Plates | 4 plates |
| Ascites PreTreat-1 | 5ml |

## STORAGE CONDITIONS

Shipped at ambient temperature. Upon arrival, store at 4°C, do NOT freeze.

## IMPORTANT

- Plates are compatible with variable speed centrifuges with rotors and carriers capable of handling stacked plates. Use speed of 500-1,000xg with a maximum of 1,000xg.
- Ensure the spin plates are balanced throughout all centrifugations with a duplicate plate filled with an appropriate volume of water.
- The plates may be used with standard vacuum manifolds and is also dependent on sample properties and sample preparation. Use at a flow rate of 2-4psi (4-8 in Hg), which is equivalent to 1-2 props per second. To preserve the quality of the resin, avoid over-drying of the resin during vacuum application.
- Each well is suitable for purifying 20-100µl serum, cell culture supernatant (with <10% fetal bovine serum (FBS)) or ascites fluid.
  ***NOTE:*** *Antibodies from FBS will co-purify with the antibody of interest.*
- Due to the mouse and rat transferrin having similar physical properties to their IgG molecules, transferrin may be detected in the IgG fraction. To eliminate the transferrin contamination it is recommend that an ammonium sulfate precipitation (See appendix) is performed before applying to the resin.
- It is recommended that ascites fluid is pretreated with Ascites PreTreat-I before purification (See appendix). Untreated ascites will be contaminant with transferrin and other abundant proteins.

- The IgG Isolation Buffer is optimized for purifying antibodies from non-hemolyzed samples. If the presence of hemoglobin hinders subsequent use of antibodies then use 10mM Tris pH8.0 in place of the IgG Isolation Buffer. Alternatively clean up sample with ammonium sulfate precipitation (See appendix).

## SPECIFICATIONS

| Species | Pearl™ IgG Purification Resin | Protein A | Protein G |
|---|---|---|---|
| Mouse | ++++ | ++++ | ++++ |
| Human | ++++ | ++++ | ++++ |
| Rat | ++++ | + | ++ |
| Hamster | ++++ | ++ | ++ |
| Guinea Pig | ++++ | ++++ | ++ |
| Rabbit | ++++ | ++++ | +++ |
| Horse | ++++ | ++ | ++++ |
| Cow | ++ | ++ | ++++ |
| Pig | ++++ | +++ | ++ |
| Sheep | ++ | + | ++ |
| Goat | ++++ | + | ++ |
| Chicken | - | - | - |

**Table 1: Performance of Pearl™ IgG Purification Resin compared to Protein A and Protein G**

## ADDITIONAL ITEMS REQUIRED

- Plate or orbital shaker
- Variable speed centrifuge with rotor and carriers capable of handling stacked plates (4.5cm height) at 500xg
- Serum Sample
- 2M NaCl

## PREPARATION BEFORE USE

1. IgG Isolation Buffer: Dissolve the entire contents of the IgG Isolation Buffer pack in 950ml DI water to produce a 100X concentrated solution. Prior to use dilute the 100X IgG Isolation Buffer 1:100 with DI water and adjust pH to 6.5 with 0.5M sodium hydroxide. For long term storage, filter the solution and store at 4°C.
2. For optimal binding of IgG, it is recommended that the serum is buffer exchanged against IgG Isolation Buffer, for this we recommend our SpinOUT™ GT-600 Spin Plate (Cat. # 786-989, 786-990).
   ***NOTE**: The serum can be diluted 10 fold with IgG Isolation Buffer, however this will dilute your final IgG solution and some loss in purification may occur.*

## PROCEDURE

1. Allow the buffers and plates to equilibrate to room temperature for at least 30 minutes.
2. Remove the seal from the bottom of the plate and place one plate on top of the wash/collection plate.
3. Remove the seal from the top of the plate.
4. Place the plate assembly in a centrifuge with a 96-well plate carrier and centrifuge at 1,000xg for 1 minute to remove the storage buffer. Discard the storage buffer.
5. Rinse the wash plate with deionized water, dry and save for future use.
6. Place the desalting plate on a new wash/collection plate and apply 20-100µl buffer-exchanged sample to the center of the resin.
   ***NOTE:*** *Touch the tip to the resin to expel all the sample. For 20µl protein samples (>300µg/ml), apply a 20µl stacker of water or buffer on top of the resin bed after the sample has fully absorbed to ensure maximal protein recovery.*
7. Incubate for 5 minutes with moderate agitation on a plate or orbital shaker.
8. Place the plate assembly in a centrifuge with a 96-well plate carrier and centrifuge at 1,000xg for 1 minute to collect the purified antibody.
   ***NOTE:*** *Discard the Pearl™ IgG Purification Spin Plate or save for future use as a balance blank.*

## APPENDIX 1: AMMONIUM SULFATE PRECIPTIATION

1. Centrifuge serum for 30 minutes at 10,000x*g* at 4°C.
2. Stir the serum and slowly, add 0.2-0.27g ammonium sulfate for every 1ml serum to produce a 35-45% final saturation.
3. Stir at 4°C for 1-4h to overnight.
4. Centrifuge at 2,000-4,000x*g* for 20 minutes at 4°C. Discard the supernatant.
5. Dissolve the precipitate in the original volume of IgG Isolation Buffer or other suitable buffer (PBS).
6. Dialyze against the same buffer at 4°C overnight with 2-3 changes of buffer to remove excess salt.

## APPENDIX2: ASCITES PRETREATMENT

1. Allow all the buffers to warm to room temperature before use.
2. Prepare the Ascites Pretreatment Solution by adding 4µl Ascites PreTreat-I to every 100µl 1X IgG Isolation Buffer required. Use 0.5ml Ascites Pretreatment Solution for every 1ml ascites fluid to be processed.
3. Transfer a known volume of ascites fluid to a centrifuge tube capable of spinning at 5,000g.
4. Place the ascites fluid on a magnetic stirrer or shaker and begin mixing. Very slowly, add the Ascites Pretreatment Solution.
   ***NOTE:*** *Adding the Ascites Pretreatment Solution to quickly or with inadequate mixing will result in precipitation of the antibodies.*

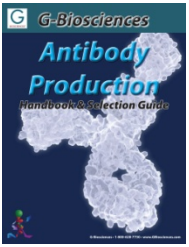
5. After adding the Ascites Pretreatment Solution, rock or rotate the sample for 10 minutes at room temperature.
6. Centrifuge the sample at 5,000g for 10 minutes. Remove the supernatant and discard the pellet.
7. Desalt the sample using our SpinOUT™ GT-600 Spin Plate (Cat. # 786-989, 786-990) equilibrated with 1X IgG Isolation Buffer.
8. Add 10µl 0.5M NaCl per 100µl sample and then purify the antibody as per the procedure above.

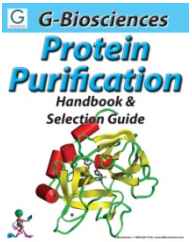
## TROUBLESHOOTING

| Issue | Possible Reason | Troubleshoot |
|---|---|---|
| No antibody detected in flow-through | No antibody present in initial sample | Examine sample by ELISA or isotyping to determine IgG presence |
| | IgG molecule failed to flow-through resin | Sample pH incorrect, ensure pH6.5-7.0 |
| | | Ensure 10M NaCl was added to sample when purifying from ascites |
| | | If using serum free media, use 1:1 resin to serum volume. |
| | Large contamination of serum proteins | Concentrate the sample with a>75K MWCO device |

## RELATED PRODUCTS

Download our Antibody Production and Protein Purification Handbooks.

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