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A Geno Technology, Inc. (USA) brand name

# Pearl™ Monoclonal IgG Purification Kit

## For the Purification of Immunoglobulin G from Ascites & Cell Culture Supernatant

## (Cat. # 786-802)

## INTRODUCTION

The Pearl™ Monoclonal IgG Purification kit allows for the rapid purification of antibodies from cell culture supernatant and ascites fluid. The 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™ Monoclonal IgG Purification kit can be used to purify antibodies direct from cell culture supernatant with less than 10% FBS or can be used with ascites fluid after treatment with the supplied Ascites PreTreat.

The Pearl™ Monoclonal IgG Purification kit can purify IgG from ~1L cell culture supernatant or 200ml ascites fluid.

## ITEM(S) SUPPLIED (Cat. # 786-802)

| Part. # | Description | Size |
|---|---|---|
| 063I-C | Pearl™ IgG Purification Resin | 200ml resin |
| 061I | IgG Isolation Buffer [100X] | For 1L |
| 354A | Ascites PreTreat-I | 5ml |
| 356A | Ascites PreTreat-II | 2ml |

*Resin is a 50% slurry in 5mM sodium phosphate, pH6.6 and 20% ethanol as a preservative.*

## STORAGE CONDITIONS

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

## IMPORTANT NOTES

- The Pearl™ IgG Purification Resin is not suitable for purification of IgY from chicken.

## 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**

## PROTOCOL 1: ANTIBODY PURIFICATION FROM ASCITES

***Additional Items Required***

Ascites Fluid Sample
Spin Purification Columns (See Related Products)
10M Sodium Chloride

***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.

***Procedure***

### Ascites pretreatment

1. Allow all the buffers to warm to room temperature before use.
2. Prepare the Ascites Pretreatment Solution by adding 40µl Ascites PreTreat-I to every 1ml 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 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. Dialyze the supernatant against 1X IgG Isolation Buffer for 2 hours with one change of dialysate or desalt the sample using our SpinOUT™ columns equilibrated with 1X IgG Isolation Buffer

### IgG Purification

1. Allow the buffers and resin to warm to room temperature before use.
2. Swirl the Pearl™ IgG Purification Resin to achieve a homogenous suspension and transfer an appropriate volume of suspension to a spin column using a wide bore pipette.
   ***NOTE:*** *For every 1ml pretreated ascites supernatant use 1ml settled resin (2ml slurry).*
3. Centrifuge the column at 1,000-5,000g for 2 minutes and discard the storage buffer. Cap the bottom of the column.
4. Add 10µl Ascites PreTreat-II for every 1ml pretreated ascites supernatant and mix.

5. Add the sample to the column and incubate for 5 minutes at room temperature with end-over-end mixing.
6. Remove the bottom cap and loosen top cap. Centrifuge the column at 1,000-5,000g for 2 minutes to collect the purified antibody. Use the antibody directly or store until required.
7. The column can be regenerated by incubating in 5 column volumes of 2M NaCl for 5 minutes, followed by five washes in 5 column volumes of IgG Isolation Buffer. Store the gel at 4°C in IgG Isolation Buffer with 0.1% sodium azide as a preservative. The column can be generated up to 3 times.

## PROTOCOL 2: PURIFICATION FROM CELL CULTURE SUPERNATANT (4-30ML)

***Additional Items Required***

Cell Culture Supernatant (4-30ml)
Spin Purification Columns (See Related Products)

***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 cell culture supernatant is dialyzed against IgG Isolation Buffer. Dialyzed against at least 100 volumes IgG Isolation Buffer or 5-10mM Sodium phosphate pH6.5-7.5 for 2 hours with at least one change of buffer.

***Procedure***

1. Allow the buffers and resin to warm to room temperature before starting the protocol.
2. Place the column in an appropriate collection tube. Swirl the Pearl™ IgG Purification Resin to achieve a homogenous suspension and transfer suspension to a column using a wide bore pipette.
   ***NOTE:*** *For every 5ml cell culture supernatant, use 2ml Pearl™ IgG Purification Resin Slurry (1ml settled resin).*
3. Centrifuge the column at 1,000-5,000g for 2 minutes and discard the storage buffer. Cap the bottom of the column.
4. Add 5ml dialyzed Cell Culture Supernatant for every 1ml settled resin. Incubate for 5 minutes at room temperature with tumbling.
5. Remove the bottom, then top, cap and centrifuge the column at 1,000-5,000g for 2 minutes to collect the purified IgG.
6. The purified IgG is now ready for downstream applications or stored.
7. The column can be regenerated by incubating in 5 column volumes of 2M NaCl for 5 minutes, followed by five washes in 5 column volumes of IgG Isolation Buffer. Store the gel at 4°C in IgG Isolation Buffer with 0.1% sodium azide as a preservative. The column can be generated up to 3 times.

## PROTOCOL 3: PURIFICATION FROM CELL CULTURE SUPERNATANT (30-1,000ML)

***Additional Items Required***

Cell Culture Supernatant (30-1,000ml)

Filter flasks, vacuum supply, filter paper, large funnel or large centrifuge tubes

***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 Cell Culture Supernatant volumes >100ml, we recommend concentrating to 100ml or less and then dialyzing.
3. For optimal binding of IgG, it is recommended that the cell culture supernatant is dialyzed against IgG Isolation Buffer. Dialyzed against at least 100 volumes IgG Isolation Buffer or 5-10mM Sodium phosphate pH6.5-7.5 for 2 hours with at least one change of buffer.
4. Ammonium sulfate precipitation prior to purification greatly enhances the purification of IgG molecules from large sample volumes. (See Appendix)

***Procedure***

1. Allow the buffers and resin to warm to room temperature before starting the protocol.
2. Swirl the Pearl™ IgG Purification Resin to achieve a homogenous suspension and transfer suspension to a suitable container using a wide bore pipette.
   ***NOTE:*** *For every 1ml cell culture supernatant, use 4ml Pearl™ IgG Purification Resin Slurry (2ml settled resin).*
3. Centrifuge the resin or use a vacuum filter to remove the storage buffer.
4. Wash the resin once with 1 volume of IgG Isolation Buffer and centrifuge the resin or use a vacuum filter to remove the buffer.
5. Combine the washed Pearl™ IgG Purification Resin with the dialyzed Cell Culture Supernatant and gently stir or agitate for 1 hr at room temperature.
6. Centrifuge the resin or use a vacuum filter to separate the resin from the supernatant. The supernatant contains the purified IgG molecules.
7. The resin can be regenerated by incubating in 5 column volumes of 2M NaCl for 5 minutes, followed by five washes in 5 column volumes of IgG Isolation Buffer. Store the gel at 4°C in IgG Isolation Buffer with 0.1% sodium azide as a preservative. The column can be generated up to 3 times.

## APPENDIX 1: AMMONIUM SULFATE PRECIPTIATION

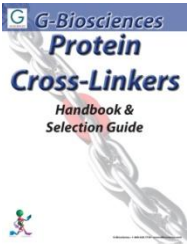
1. Centrifuge serum for 30 minutes at 10,000x*g* at 4°C.
2. Stir the serum and slowly, add 0.2-0.27g ammonium sulfate for every 1ml serum to produce a 35-45% final saturation.
3. Stir at 4°C for 1-4h to overnight.
4. Centrifuge at 2,000-4,000x*g* for 20 minutes at 4°C. Discard the supernatant.
5. Dissolve 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.

## 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 Protein Cross-linkers Handbook.




http://info.gbiosciences.com/complete-protein-cross-linkers-handbook/

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