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A Geno Technology, Inc. (USA) brand name

# Pearl<sup>™</sup> IgG Purification (Spin format)

### For the Purification of Immunoglobulin G from Serum

(Cat. # 786-798)



#### **INTRODUCTION**

The Pearl<sup>™</sup> IgG Purification (Spin format) kit allows for the one-step purification of immunoglobulin G from serum. 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<sup>™</sup> IgG Purification (Spin format) kit can purify up to 25mg IgG.

#### KIT COMPONENTS (Cat. # 786-798)

Description	Size	
Pearl <sup>™</sup> IgG Purification Resin	3ml resin	
IgG Isolation Buffer [100X]	For 1L	
Spin Columns, 1ml	20	
Caps	20	

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**

 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.

#### **SPECIFICATIONS**

Species	Pearl <sup>™</sup> 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<sup>™</sup> IgG Purification Resin compared to Protein A and Protein G

#### **ADDITIONAL ITEMS REQUIRED**

- Serum Sample
- 2M NaCl

#### **PREPARATION BEFORE USE**

- 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.
- For optimal binding of IgG, it is recommended that the serum is dialyzed against IgG Isolation Buffer, for this we recommend our Tube-O-DIALYZER (Cat. # 786-610 to 786-624). Dialyzed against at least 100 volumes IgG Isolation Buffer or 5-10mM Sodium phosphate pH6.5-7.5 with at least two changes of buffer. 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 resin to warm to room temperature before starting the protocol.
- 2. Snap off the bottom end cap of the column and retain. Place the column in a collection tube.
- Swirl the Pearl<sup>™</sup> IgG Purification Resin to achieve a homogenous suspension and transfer 500µl of suspension to a column using a wide bore pipette. NOTE: For every 10-100µl serum use 200µl Pearl<sup>™</sup> IgG Purification Resin Slurry (100µl settled resin). For serum samples diluted 10-fold with IgG Isolation Buffer up to 500µl diluted serum can be used with 100µl settled resin.
- 4. Centrifuge the spin column at 2,000-5,000xg for 1 minute. Discard the flow-through.
- 5. Add 300µl IgG Isolation Buffer to the column.
- 6. Briefly centrifuge (10-30 seconds) and discard the flow through. Repeat steps 4 and 5 once.
- Add 100-500µl diluted serum sample or 10-100µl dialyzed (buffer-exchanged) serum to the column and seal the column with the end cap from step 2. Incubate for 5 minutes at room temperature with tumbling.
- 8. Remove the bottom, then top, cap and centrifuge the column for 1 minute to collect the purified IgG.
- 9. The purified IgG is now ready for downstream applications or stored.
- 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.

#### **APPENDIX 1: AMMONIUM SULFATE PRECIPTIATION**

- 1. Centrifuge serum for 30 minutes at 10,000xg 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 2,000-4,000xg 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.

#### **RELATED PRODUCTS**

Download our Antibody Production and Protein Purification Handbooks.



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