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A Geno Technology, Inc. (USA) brand name

Pearl™ IgG Purification Resin

For the Purification of Immunoglobulin G from Serum

(Cat. # 786-800, 786-801)

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INTRODUCTION

The Pearl™ IgG Purification Resin 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.

ITEM(S) SUPPLIED

Cat. #	Description	Size
786-800	Pearl™ IgG Purification Resin	3ml resin
786-801	Pearl™ IgG Purification Resin	25ml resin

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

Table 1: Relative affinity of Pearl™ IgG Purification Resin compared to Protein A and Protein G

PROTOCOL 1: GRAVITY FLOW

Additional Items Required

- Serum Sample
- Isolation Buffer: 5mM Sodium Phosphate, pH6.5
- 2M NaCl
- Columns

Preparation Before Use

1. For optimal binding of IgG, it is recommended that the serum is dialyzed against Isolation Buffer, for small sample volumes (<2.5ml) we recommend our Tube-O-DIALYZER (Cat. # 786-610 to 786-624). Dialyzed against at least 300 volumes Isolation Buffer with at least two changes of buffer.
   NOTE: *The serum can be diluted 10 fold with Isolation Buffer, however this will dilute your final IgG solution and some loss in purification may occur.*

Procedure

1. Allow all the buffers 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 column using a wide bore pipette.
   NOTE: *For every 1-2ml serum use 1ml settled resin (2ml 50% slurry).*
3. Allow the column to drain and then add 10 volumes of settled resin of Isolation Buffer. For every 1ml resin use a total of 10ml Isolation Buffer. Allow the buffer to freely flow through the column.
4. Add the serum sample and allow to flow through the column. Collect the flow-through containing the IgG in 0.5-1ml fractions.
   NOTE: *IgG will begin to emerge after the void volume, which is ~70% the resin bed volume. The emergence can be monitored with UV absorbance at 280nm or with a protein assay.*
5. After the serum has passed through the column, add 0.5-1ml Isolation Buffer to elute the IgG in the resin bead. Monitor elution at 280nm and continues adding 0.5-1ml Isolation Buffer until the level of protein has reached a baseline.
6. Combine the appropriate fractions. 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 Isolation Buffer. Store the gel at 4°C in Isolation Buffer with 0.1% sodium azide as a preservative. The column can be generated up to 3 times.

PROTOCOL 2: SPIN COLUMN

Additional Items Required

- Serum Sample
- Isolation Buffer: 5mM Sodium Phosphate, pH6.5
- Spin columns (See Related Products)

Preparation Before Use

1. For optimal binding of IgG, it is recommended that the serum is dialyzed against Isolation Buffer, for this we recommend our Tube-O-DIALYZER (Cat. # 786-610 to 786-624). Dialyzed against at least 100 volumes 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 Isolation Buffer, however this will dilute your final IgG solution and some loss in purification may occur.*

Procedure

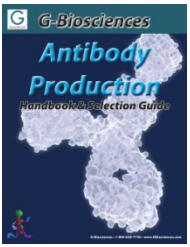
1. Allow the buffers and resin to warm to room temperature before starting the protocol.
2. Swirl the Pearl™ IgG Purification Resin to achieve a homogenous suspension and transfer an appropriate volume of suspension to a column using a wide bore pipette.
   NOTE: *For every 10-100µl serum use 200µl Pearl™ IgG Purification Resin Slurry (100µl settled resin). For serum samples diluted 10-fold with Isolation Buffer up to 500µl diluted serum can be used with 100µl settled resin.*
3. Place the column in a collection tube and centrifuge the spin column at 2,000-5,000x*g* for 1 minute. Discard the flow-through.
4. Add one column volume of Isolation Buffer to the column.
5. Briefly centrifuge (10-30 seconds) and discard the flow through. Repeat steps 4 and 5 once.
6. Add 100-500µl diluted serum sample or 10-100µl dialyzed (buffer-exchanged) serum for every 100µl settled resin to the column and seal the column. Incubate for 5 minutes at room temperature with tumbling.
7. Remove the bottom, then top, cap and centrifuge the column for 1 minute to collect the purified IgG.
8. The purified IgG is now ready for downstream applications or stored.
9. 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 Isolation Buffer. Store the gel at 4°C in Isolation Buffer with 0.1% sodium azide as a preservative. The column can be generated up to 3 times.

APPENDIX 1: AMMONIUM SULFATE PRECIPITATION

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 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 Handbook.



http://info.gbiosciences.com/complete-Antibody-Production-handbook/

For other related products, visit our website at www.GBiosciences.com or contact us.

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