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A Geno Technology, Inc. (USA) brand name

Pearl™ IgG Purification Kit

For the Purification of Immunoglobulin G from Serum

(Cat.# 786-799)

INTRODUCTION

Pearl™ IgG Purification 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™ IgG Purification kit can purify IgG from ~100ml serum, depending on the procedure used.

IMPORTANT

For a spin-column format: For every 10-100µl serum use 200µl Pearl™ 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.

For gravity flow column format: Apply 1-2ml serum for every 1ml settled resin (2ml slurry). Ensure the appropriate volume of resin is used as too little will result in contamination with other serum proteins and too much may result in loss of IgG.

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.

ITEMS SUPPLIED (Cat. # 786-799)

Description	Size	Part #
Pearl™ IgG Purification Resin	25ml resin	063I-B
IgG Isolation Buffer [100X]	For 1L	061I

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.

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: GRAVITY FLOW

Additional Items Required

Serum Sample
Gravity flow columns (Medi column, 7ml total volume (Cat. # 786-169); Maxi column, 23ml total volume (Cat. # 786-197))

PREPARATION BEFORE USE

1. For optimal binding of IgG, it is recommended that the serum is dialyzed against IgG 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 IgG Isolation Buffer 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.
2. 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

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 1X IgG Isolation Buffer. For every 1ml resin use a total of 10ml IgG 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 IgG Isolation Buffer to elute the IgG in the resin bead. Monitor elution at 280nm and continues adding 0.5-1ml IgG 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 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: SPIN COLUMN

Additional Items Required

Serum Sample
Spin columns (See Related Products)

PREPARATION BEFORE USE

1. 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.
2. 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

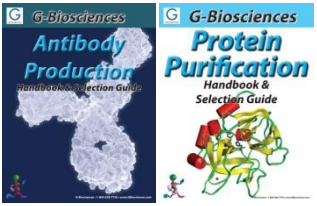
1. Allow the buffers and resin to warm to room temperature before starting the protocol.
2. Swirl the Pearl™ IgG Purification Resin to achieve a homogenous suspension and transfer an appropriate volume of suspension to a column using a wide bore pipette.
   NOTE: For every 10-100µl serum use 200µl Pearl™ IgG Purification Resin Slurry (100µl settled resin). For serum samples diluted 10-fold with IgG 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 IgG 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 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.

RELATED PRODUCTS

Download our Antibody Production and Protein Purification Handbook.



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