Pearl™ Antibody Clean-Up Kit

For the Removal of BSA, Gelatin and amine buffers to enable antibody labeling and conjugation

(Cat. # 786-803, 786-1603)

INTRODUCTION

Commercially available antibodies are mostly stored in buffers containing antibody stabilizers, including BSA and Gelatin, or other amine containing buffering agents, including tris, that may interfere in downstream applications of antibodies. These proteins and buffering agents can interfere with labeling and conjugation processes, including biotinylation, fluorescent dye labeling, covalent antibody immobilization and other applications.

The Pearl™ Antibody Clean Up kit is designed for the rapid clean up of antibody solutions to ensure the antibody is in an optimal buffer and free from interfering agents. The Pearl™ IgG Purification Resin binds the high abundant, non-IgG proteins (i.e. BSA and gelatin) and allows the IgG molecules to pass through in a physiological buffer.

The Pearl™ Antibody Clean Up kit is designed to purify samples containing <0.5% BSA and gelatin. For samples containing >0.5 % BSA or gelatin, increase the amount of Pearl IgG Purification Resin-I proportionally.

ITEM(S) SUPPLIED

Description	**Cat. #786-803 (10 X 100 µl Samples)**	**Cat. #786-1603 (10 x 1ml Sample)**
Pearl™ IgG Isolation Buffer	250 ml	2 x 250 ml
Pearl™ IgG Purification Resin-I	1.25 ml resin	10.5 ml resin
Pearl™ Binding Agent	1ml	1 ml
Spin Column, 1ml	10	-
Spin Column, 3 ml	-	10
SpinOUT™ GT-600, 1ml	10/bag	-
SpinOUT™ GT-600, 5ml	-	10/bag
Caps (Micro)	10	-
Caps (Medi)	-	10
Rubber Stoppers	10	10

Pearl™ IgG Purification Resin-I is a 50% slurry in 5mM sodium phosphate, pH6.6, 50% glycerol and 0.05% Azide.

STORAGE CONDITIONS

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

ADDITIONAL ITEMS REQUIRED

- 2 ml collection tubes
- 15 ml collection tubes

IMPORTANT INFORMATION

- The Pearl™ Antibody Clean Up kit (Cat. # 786-803) comes with 1 ml SpinOUT™ GT-600 columns for desalting and 1 ml spin columns for antibody purification with Pearl resin. Pearl™ Antibody Clean Up kit (Cat. # 786-1603) comes with 5 ml SpinOUT™ GT-600 columns for desalting and 3 ml spin columns for antibody purification with Pearl resin
- Stoppers for Spin Columns included in the kit.
- For higher concentrations of BSA (>0.5%), increase the amount of Pearl™ IgG Purification Resin-I proportionally.
- Bring all components to room temperature before use.

PROTOCOL

For purification of antibody sample in PBS containing 0.5% BSA, 50% glycerol and azide (0.02-0.05%)

Ensure the IgG Isolation Buffer and the Pearl™ IgG Purification Resin-I are equilibrated to room temperature before starting the protocol.

Sample preparation:

1. Centrifuge the tube briefly to bring the antibody solution to the bottom of tube.
2. Add 10 µl of Pearl™ Binding Agent to 100 µl antibody sample (Cat. # 786-803) [1µl/10µl] and 100 µl of Pearl™ Binding Agent to 1ml antibody sample (Cat. # 786-1603).
3. Vortex the microfuge tube to mix antibody sample with Pearl™ Binding Agent.

IgG Purification

1. Swirl the Pearl™ IgG Purification Resin-I to achieve a homogenous suspension and transfer 200µl of suspension (Cat. # 786-803) to a column using a wide bore pipette.
   NOTE: *For purification of 1ml of sample (Cat. # 786-1603) use 2ml of* Pearl™ IgG *Purification Resin-I suspension.*
2. Remove the end cap from the spin column. 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.
3. Add three bed volume (BV) of Pear™ l IgG Isolation Buffer to the column.
4. Briefly centrifuge (10-30 seconds) and discard the flow through. Repeat steps 3 and 4 once.
5. Seal the bottom of column with rubber stopper and add 100 µl (Cat. # 786-803) or 1 ml (Cat. #786-1603) of Pearl™ Binding Agent treated antibody solution (Step 3 of sample preparation) to the column.

6. Seal the top of column with the cap and incubate the column for 5 minutes at room temperature with tumbling.
   NOTE: *For samples with >1% BSA/gelatin, adjust the volume of resin used to ensure sufficient resin is available to bind the stabilizer proteins.*
7. Remove the bottom, then top, cap and centrifuge the column at 2,000-5,000x*g* for 1 minute to collect the sample.
8. Seal the bottom of column and reload the sample to column and repeat step 6 and 7 once to collect the purified IgG sample.
   NOTE: *The purified IgG is now ready for downstream applications or stored. The antibody can be evaluated by SDS-PAGE to determine the presence of BSA or gelatin*

For purification of antibody sample containing tris (or other amine buffer), 0.5% BSA, 50% glycerol and azide (0.02-0.05%)

Desalting

1. Centrifuge the SpinOUT™ column at 1,000g for 2 minutes to compact the resin.
2. Prepare the Spin-*OUT*™ column by removing the top and then bottom caps. Place into an appropriate collection tube.
3. Mark one side of the column and ensure in all centrifugations the mark is facing outwards during centrifugation.
4. Centrifuge the column at 1,000g for 2 minutes to remove the storage buffer.
5. Place the column in a new collection tube (2 ml) and remove the cap.
6. Add 0.3 ml of Pearl™ IgG Isolation Buffer to the column.
   NOTE: *For 5 ml SpinOUT™ GT-600 use 15 ml collection tube and 1.5 ml Pearl™ IgG Isolation for washes.*
7. Centrifuge the column at 1,000g for 2 minutes to remove the buffer.
8. Repeat steps 6 and 7 two more times, ensuring the buffer is discarded after each centrifugation.
9. Centrifuge the column at 1,000 g for 2 minutes to remove residual buffer
10. Place the column in a new collection tube and remove the cap.
11. Slowly, apply 100 µl antibody solution to the centre of column (Cat. # 786-803) or 1 ml antibody solution to the centre of column (Cat. # 786-1603).
12. Centrifuge the column at 1,000g for 4 minutes to collect the desalted antibody solution. Discard the column.

IgG Purification

1. Ensure the Pearl™ IgG Isolation Buffer and the Pearl™ IgG Purification Resin-I are equilibrated to room temperature before starting the protocol.
2. Swirl the Pearl™ IgG Purification Resin-I to achieve a homogenous suspension and transfer 200µl of suspension (Cat. # 786-803) to a column using a wide bore pipette.
   NOTE: *For purification of 1ml of sample (Cat.# 786-1603) use 2ml of* Pearl™ IgG *Purification Resin-I suspension.*

3. Remove the end cap from the spin column. 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 three BV of Pearl™ IgG Isolation Buffer to the column.
5. Briefly centrifuge (10-30 seconds) and discard the flow through. Repeat steps 4 and 5 once.
6. Seal the bottom of column with rubber stopper and add 100 µl (Cat. # 786-803) or 1 ml (Cat. #786-1603) desalted antibody solution (Step 10 above).
7. Seal the top of column with the cap and incubate the column for 5 minutes at room temperature with tumbling.
   NOTE: *For samples with >1% BSA/gelatin, adjust the volume of resin used to ensure sufficient resin is available to bind the stabilizer proteins.*
8. Remove the bottom, then top, cap and centrifuge the column at 2,000-5,000x*g* for 1 minute to collect the sample.
9. Seal the bottom of column with rubber cap. Reload the sample to column and repeat step 7 and 8 once to collect the purified IgG sample.
   NOTE: *The purified IgG is now ready for downstream applications or stored. The antibody can be evaluated by SDS-PAGE to determine the presence of BSA or gelatin*

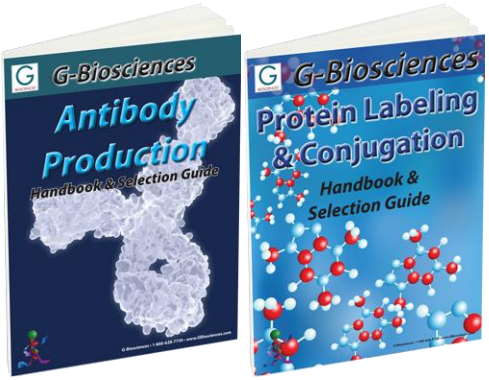
TROUBLESHOOTING

Issue	Suggested reason	Possible solution
*BSA/Gelatin bands present on stained SDS-PAGE gel	Column was overloaded with sample	Use fresh Pearl™ IgG Purification Resin-I and repeat the procedure with same sample
	Sample contains salts greater than 25 mM and or pH is not neutral	Dialyze sample against Pearl™ IgG Isolation Buffer or perform buffer exchange using desalting column
No antibody was detected in purified sample	Sample was devoid of antibody	Ensure by other means such as ELISA or isotyping kit that the sample contains antibody

* Small amount of BSA and gelatin when compared to IgG fraction does not interfere with labeling and conjugation reactions.

RELATED PRODUCTS

Download our Antibody Production and Protein Labeling and Conjugation Handbooks.



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

http://info2.gbiosciences.com/complete-protein-labeling-conjugation-handbook

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

www.GBiosciences.com