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Remove BSA and gelatin from antibody solutions using Melon™ Gel

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Introduction

Bovine serum albumin (BSA) and gelatin are commonly added to purified antibody solutions as stabilizers for long-term storage. Inclusion of 0.2-1.0% (2-10mg/mL) BSA or gelatin helps to stabilize antibody solutions that are < 1mg/mL, and generally does not interfere with immunodetection methods. However, the protein additives, which are usually present at a concentration greater than the antibody protein itself, will interfere with most biotinylation, fluorescent labeling, and covalent immobilization methods involving the antibody. For such methods, the antibody must be affinity purified from the stabilizing proteins.

Three basic strategies exist for removing BSA and gelatin from antibody samples. Immobilized Cibacron* Blue F3GA Dye may be used to bind and deplete BSA from serum samples (e.g., Thermo Scientific Pierce* Blue Albumin Removal Kit, Product No. 89845); however, this ligand does not have a high binding capacity for BSA and it can also bind IgG. Alternatively, Protein A and Protein G Agarose (see Related Products) specifically bind antibody, allowing removal of the BSA and gelatin before eluting the antibody to recover it in purified form. Unfortunately, this method usually involves harsh elution conditions that may adversely affect antibody function; furthermore, the recovered antibody must be thoroughly dialyzed or desalted to exchange it into a buffer that is compatible with the subsequent labeling or conjugation experiment.

A third method, which is described in this Tech Tip, involves using the Thermo Scientific Melon* Gel Support to bind and remove BSA and/or gelatin. Melon Gel Kits were developed to purify antibodies from serum, cell culture supernatant, and ascites fluid, and any one of the kits (see Related Products Section) can be adapted for removal of BSA or gelatin from purified antibody stock solutions. In fact, the Antibody Clean-up Kit (Product No. 44600) is a Melon Gel kit specifically for designed for this application. This Tech Tip provides a general description of the BSA-removal protocol for those who might already possess one of the Melon Gel Antibody Purification Kits. For samples that have been diluted or exchanged into the supplied mild, physiological pH, non-amine Purification Buffer, Melon Gel binds non-antibody proteins, such as albumin and transferrin, allowing the antibody to flow through in a purified form that is ready for storage and downstream applications.

Important Product Information

- Melon Gel Support binds albumin from most common sources including human, mouse, rabbit, rat, goat, and bovine.
- For the spin-column format, 100µL of settled gel has the capacity to bind up to 8mg of bovine serum albumin. A capacity for albumin from other sources or gelatin has not been determined.

Spin column Procedure for BSA/Gelatin Removal using the Melon Gel Support

A. Additional Materials Required

- Melon Gel IgG Spin Purification Kit (e.g., Product No. 45206)
- Microcentrifuge set to moderate speed (2000-6000 × *g*). Centrifugal force > 6000 × *g* produces suboptimal results.
- 1000µL, 200µL, and 10µL pipettors and pipette tips, including one large orifice tip for dispensing gel slurry
- Thermo Scientific Zeba* Desalt Spin Columns (0.5mL, Product No. 89882 or 2mL, Product No. 89889) or Slide-A-Lyzer* Dialysis Cassettes or MINI Units (e.g., 66385 or 69576, respectively; see Related Products).
- End-over-end rocker or rotator

B. Protocol for BSA/Gelatin Removal

Note: 100µL of settled Melon Gel has the capacity to bind up to 8mg of BSA. Therefore, 100µL of settled Melon Gel can be used to process at least 500µL of antibody sample that contains 1% BSA (= 5mg). Be aware that the Pierce Mini-Spin Columns in the kit have a maximum volume capacity of ~600µL.

1. Dilute antibody sample 1:10 in Melon Gel Purification Buffer, or to avoid diluting, perform a buffer exchange with 1X Melon Gel Purification Buffer using a desalting column or dialysis unit.
2. Equilibrate the Melon Gel IgG Purification Support and Purification Buffer to room temperature (~15 minutes).
3. Swirl bottle containing the Purification Support (do not vortex) to obtain an even suspension. To ensure proper gel slurry dispensing, use a wide orifice or cut pipette tip to dispense 500µL of slurry into a Mini-Spin Column placed in a microcentrifuge tube. Swirl the bottle of gel slurry before pipetting each sample to maintain the gel suspension.
4. Centrifuge the uncapped column/tube assembly for 1 minute, then remove the spin column and discard flow-through.

   Note: Perform all centrifugations at 2000-6000 × *g*. Centrifugal force > 6000 × *g* produces suboptimal results.
5. Add 300µL of Purification Buffer to the column, pulse centrifuge for 10 seconds and discard flow-through. Repeat this wash once. Place the bottom cap on the column.
6. Add up to 500µL of diluted or buffer-exchanged antibody sample containing 1% BSA. Cap the column and incubate for 5 minutes at room temperature with end-over-end mixing.

   Note: If concentrations of BSA/gelatin are > 1%, adjust the amount of gel or the amount of sample applied to the gel to ensure that a sufficient amount of gel is used to bind all the stabilizer protein.
7. Remove bottom cap from the column, loosen the top cap, and re-insert spin column in the collection tube.
8. Centrifuge for 1 minute to collect the purified antibody in the microcentrifuge tube.
9. Evaluate purity of the sample by SDS-PAGE. If the BSA/gelatin has been successfully removed, use antibody directly for downstream applications or store it as desired. If some BSA/gelatin is still present in the sample, repeat purification with new or regenerated Melon Gel Support.
10. Discard the used gel support. If the gel must be used again, it can be regenerated by adding 500µL of 5M NaCl or 0.5N NaOH, mix for 5 minutes, centrifuge, and discard the flow-through. Wash the gel five times by adding 500 µl of Purification Buffer, centrifuge and discard the flow-through. Add 500µL of Purification buffer and store at 4ºC. The gel may be regenerated three times without significant loss of selectivity. For storage longer than 1 week, add a final concentration of 0.02% sodium azide to the 1X Melon Gel Purification Buffer used to wash the column.

Troubleshooting

Problem	Possible Cause	Solution
No antibody detected in any flow-through fraction by absorbance at 280nm	Sample devoid of antibody	Ensure by other means, e.g., ELISA or isotyping kit, that the sample contains IgG.
	Antibody of interest bound to gel and did not flow through	Ensure the sample pH is 6.5-7.0
BSA/gelatin bands present on stained SDS-polyacrylamide gel	Sample contains salts > 25mM and/or pH is not neutral	Dialyze sample against the Purification Buffer or perform a buffer exchange using a desalting column
		Ensure the sample pH is 6.5-7.0
		If using gel that was regenerated, thoroughly wash gel to remove all Regenerant and then equilibrate gel with 1X Purification Buffer
	Centrifugations were performed at > 6000 × *g*	Perform all centrifugation steps at 2000-6000 × *g*
	Column was overloaded	Repeat the procedure with the same sample and new or regenerated gel

Related Thermo Scientific Pierce Products

45206	**Melon Gel IgG Spin Purification Kit,** includes 3mL of Melon Gel and supplies for purification of antibody from 25 × 100µL serum samples
45212	**Melon Gel IgG Purification Kit,** includes 25ml of Melon Gel and supplies for purification of 100mg antibody (10mL serum with 5 regenerations)
45214	**Melon Gel Monoclonal IgG Purification Kit,** includes 200mL of Melon Gel and supplies for purification of antibody from 1L of cell culture supernatant or 200mL ascites
89932	**Melon Gel Chromatography Cartridge,** 2 × 1mL cartridges
89932	**Melon Gel Chromatography Cartridge,** 1 × 5mL cartridge
89889	**Zeba Spin Desalting Column, 2mL,** 5 columns for processing 200-700µL samples
89882	**Zeba Spin Desalting Column, 0.5mL,** 5 columns for processing 30-130µL samples
66385	**Slide-A-Lyzer Dialysis Cassette Kit**, 10 dialysis cassettes, each appropriate for 0.5-3mL samples
69576	**Slide-A-Lyzer MINI Dialysis Units**, 10 units and float, each appropriate for 10-100µL samples
45200	**NAb* Protein A Spin Purification Kit**
45201	**NAb* Protein G Spin Purification Kit**
44894	**AminoLink* Plus Immobilization Kit**
21435	**EZ-Link* Sulfo-NHS-LC-Biotinylation Kit**

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Current versions of product instructions are available at *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.