

Remove BSA and gelatin from antibody solutions using Melon™ Gel

TR0055.1

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% (2-10 mg/ml) BSA or gelatin helps to stabilize antibody solutions that are < 1 mg/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., SwellGel® 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.

The third method, which is described in this Tech Tip, involves using the Melon™ Gel IgG Spin Purification Kit (Product No. 45206) to bind and remove BSA and gelatin. The Melon Gel Kit was developed to purify antibodies from serum, cell culture supernatant, and ascites fluid. 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, 100 µl of settled gel has the capacity to bind up to 8 mg 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 (Product No. 45206)
- Microcentrifuge set to moderate speed (2,000-6,000 × g). Centrifugal force > 6,000 × g produces suboptimal results.
- 1,000 µl, 200 µl, and 10 µl pipettors and pipette tips, including one large orifice tip for dispensing gel slurry
- Zeba™ Desalt Spin Columns (0.5-ml, Product No. 89882 or 2-ml, 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: 100 μ l of settled Melon Gel has the capacity to bind up to 8 mg of BSA. Therefore, 100 μ l of settled Melon Gel can be used to process at least 500 μ l of antibody sample that contains 1% BSA (= 5 mg). Be aware that the Pierce Mini-Spin Columns in the kit have a maximum volume capacity of \sim 600 μ l.

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 (\sim 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 500 μ l 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 2,000-6,000 \times g. Centrifugal force $>$ 6,000 \times g produces suboptimal results.
5. Add 300 μ l 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 500 μ l 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 500 μ l of 5 M NaCl or 0.5 N NaOH, mix for 5 minutes, centrifuge, and discard the flow-through. Wash the gel five times by adding 500 μ l of Purification Buffer, centrifuge and discard the flow-through. Add 500 μ l of Purification buffer and store at 4°C. 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 280 nm	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 $>$ 25 mM 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 $>$ 6,000 \times g	Perform all centrifugation steps at 2,000-6,000 \times g
	Column was overloaded	Repeat the procedure with the same sample and new or regenerated gel

Related Pierce Products

89889	Zeba Desalt Spin Column, 2 ml , 5 columns for processing 200-700 µl samples
89882	Zeba Desalt Spin Column, 0.5 ml , 5 columns for processing 30-130 µl samples
66385	Slide-A-Lyzer Dialysis Cassette Kit , 10 dialysis cassettes, each appropriate for 0.5-3 ml samples
69576	Slide-A-Lyzer MINI Dialysis Units , 10 units and float, each appropriate for 10-100 µl samples
25200-25244	Precise™ Protein Gels (see catalog or web site for a complete listing)
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

Patent pending on Melon™ Gel Technology.

Slide-A-Lyzer® Dialysis Cassette is protected by U.S. Patent No. 5,503,741.

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

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