INSTRUCTIONS

Pierce™ Antibody Clean-up Kit

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44600

Number	Description
44600	**Pierce Antibody Clean-up Kit,** sufficient reagents for up to 10 purifications of 0.5mL IgG samples containing 1% BSA
	Kit Contents:
	Melon™ Gel IgG Purification Support, 1.2mL of settled gel supplied as 20% slurry (i.e., 6mL total volume); contains 0.02% sodium azide
	Melon Gel Purification Buffer, 100mL; contains 0.02% sodium azide
	Spin Columns, 10 each, 0.8mL spin columns with white caps and bottom plugs
	Microcentrifuge Tubes, 30 each, 2.0mL spin column collection tubes
	Zeba™ Spin Desalting Columns, 2mL, 10 each, for 200-700µL samples
	Storage: Upon receipt store at 4°C. Product shipped at ambient temperature.

Introduction

The Thermo Scientific Pierce Antibody Clean-up Kit is a complete kit for removing bovine serum albumin (BSA) and gelatin (up to 1%) from IgG samples. Stabilizing proteins are commonly added to purified antibody solutions for long-term storage. Including 0.2-1.0% (2-10mg/mL) BSA or gelatin helps stabilize antibody solutions that are < 1mg/mL and generally does not interfere with immunodetection methods; however, stabilizing proteins, which are usually present at a concentration greater than the antibody, will interfere with most biotinylation, fluorescent dye labeling, antibody fragmentation and covalent immobilization methods. For such methods, the antibody must be isolated from stabilizing proteins.

The Melon Gel IgG Purification Support included in this kit binds non-antibody proteins, such as BSA and gelatin, allowing the antibody to flow through in a purified form that is ready for storage and downstream applications. The Thermo Scientific Zeba Spin Desalting Columns contain an exclusive high-performance resin that offers exceptional desalting, ensuring that the IgG sample is in the optimal buffer for efficient purification.

Important Product Information

- Melon Gel Support binds albumin from most common sources including human, mouse, rabbit, rat, goat, and bovine. Albumin binding from other sources has not been determined.
- The Melon Gel has the capacity to bind up to 8mg of bovine serum albumin per 100µL of settled gel. Therefore, 100µL of settled Melon Gel can be used to process at least 500µL of antibody sample that contains 1% BSA (= 5mg). The spin columns included in the kit have a maximum capacity of 800µL.

Additional Materials Required

- 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 Melon Gel
- 15mL conical collection tubes
- End-over-end rocker or rotator

Procedure for BSA/Gelatin Removal

Note: For samples of less than 200µL, use Zeba Spin Desalting Columns, 7K MWCO, 75µL for 2-12µL samples (Product No. 89877) or 0.5mL for 30-130µL samples (Product No. 89882).

A. Desalting

1. Twist off the bottom closure (SAVE for later use) of a Zeba Spin Desalting Column and loosen cap. Place column in a 15mL collection tube.
2. Centrifuge column at 1000 × *g* for 2 minutes to remove storage solution. Place a mark on the side of the column where the compacted resin is slanted upward. Place column in centrifuge with the mark facing outward in all subsequent centrifugation steps.

   Note: Resin will appear compacted after centrifugation.
3. Add 1mL of Melon Gel Purification Buffer to column. Centrifuge at 1000 × *g* for 2 minutes and discard flow-through. Repeat this step three additional times, discarding buffer from the collection tube.
4. Place column in a new collection tube, remove cap and slowly apply sample to the center of the compacted resin bed.
5. Replace cap and centrifuge at 1000 × *g* for 2 minutes to collect the sample. Discard the column after use.

B. BSA/Gelatin Removal

1. Equilibrate the Melon Gel IgG Purification Support and Purification Buffer to room temperature (~15 minutes).
2. Swirl bottle (do not vortex) containing the Purification Support 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 Spin Column placed in a microcentrifuge tube. Swirl the bottle of gel slurry before pipetting each sample to maintain the gel suspension.

   Note: For samples < 100µL in volume, use a volume of settled resin that is equal to the volume of the sample. After centrifuging and collecting the purified IgG, spin additional Melon Gel Purification equal to one-half the sample volume through the column and collect it in the same microcentrifuge tube to ensure recovery of all of the IgG. For example, if the sample volume is 25µL, use 25µL of settled resin or 125µL of 20% slurry. After centrifuging to collect the IgG, add another 12.5µL of Melon Gel Purification Buffer and centrifuge, collecting this buffer in the same microcentrifuge as the IgG.

   Note: Do not process samples < 20µL with this kit. For samples < 30µL, use two of the 75µL Zeba Spin Desalting Columns and pool the buffer-exchanged IgG before processing with the Melon Gel.
3. Centrifuge the uncapped column/tube assembly for 1 minute, then remove the spin column and discard the flow-through.

   Note: Perform all centrifugations at 2,000-6,000 × *g*. Centrifugal force > 6,000 × *g* produces suboptimal results.
4. Add 300µL of Melon Gel Purification Buffer to the column, pulse centrifuge for 10 seconds and discard flow-through. Repeat this wash once. Reseal the column by inverting the original snap-off closure, and with a slight twisting motion, press it firmly to the bottom tip of the column.
5. Add up to 500µL of buffer-exchanged antibody sample containing 1% BSA or gelatin. 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 Melon Gel or the amount of sample applied to ensure that a sufficient amount of Melon Gel is used to bind all of the stabilizer protein.
6. Remove bottom cap from the column, loosen the top cap, and re-insert spin column in the collection tube.
7. Centrifuge for 1 minute to collect the purified antibody in the microcentrifuge tube.

8. 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.
9. Discard the used Melon Gel. 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 Melon Gel five times by adding 500µL of Purification Buffer, centrifuge and discard the flow-through. Add 500µL of Purification buffer, cap the top of the column, then reseal the bottom by inverting the original snap-off closure, and with a slight twisting motion, press it firmly to the bottom tip of the column,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 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 was 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 flowthrough.	Ensure the sample pH is 6.5-7.0.
BSA/gelatin bands present on stained SDS-polyacrylamide gel	Sample contained 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 Melon 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 × *g*.	Perform all centrifugation steps at 2,000-6,000 × *g*.
	Column was overloaded.	Repeat the procedure with the same sample and new or regenerated Melon Gel.

Related Thermo Scientific Products

89889 **Zeba Spin Desalting Column, 7K MWCO, 2mL,** 5 columns

89868 **Pierce Centrifuge Columns, 0.8mL,** 50 units

89956 **NAb Protein A Spin Columns, 1mL,** 5 × 1mL

44988 **Pierce F(ab´)$_2$ Preparation Kit**

44688 **Pierce F(ab´)$_2$ Micro Preparation Kit**

44985 **Pierce Fab Preparation Kit**

44685 **Pierce Fab Micro Preparation Kit**

44980 **Pierce IgG$_1$ Fab and F(ab´)$_2$ Preparation Kit**

44680 **Pierce IgG$_1$ Fab and F(ab´)$_2$ Micro Preparation Kit**

45206 **Melon Gel IgG Spin Purification Kit**

XP04200BOX **Novex™ 4-20% Tris-Glycine Protein Gels** (see thermofisher.com/proteingels for a complete listing)

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition. The information in this guide is subject to change without notice.

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Revision	Date	Description
C	31 July 2024	Correcting spin column usage.
B	05 January 2018	Correcting protocol.
A	03 May 2017	New document for Pierce™ Antibody Clean-up Kit.