INSTRUCTIONS

Melon™ Gel IgG Purification Kit

45212

1512.7

Number	Description
45212	**Melon Gel IgG Purification Kit,** sufficient reagents to purify IgG from 75-150mL of serum, depending on the procedure used
	Kit Contents:
	Melon Gel IgG Purification Support, 25mL of settled gel, supplied as 20% slurry (i.e., 125mL total volume)
	Melon Gel Purification Buffer, supplied as a dry mix that makes 1L of 100X buffer when reconstituted
	Melon Gel Regenerant, supplied as a dry mix that makes 1L when reconstituted
	Storage: Upon receipt store at 4°C. Product shipped at ambient temperature.

Table of Contents

Introduction

The Melon Gel IgG Purification System purifies antibodies from serum by removing non-relevant proteins often present in high abundance. The Melon Gel IgG Purification Support binds non-antibody serum proteins, such as albumin and transferrin, using physiological pH allowing the antibody to flow through in a mild buffer suitable for storage and downstream applications. Serum samples can be applied directly to Melon Gel without the need for ammonium sulfate precipitation, provided the sample is not hemolyzed.

The Melon Gel IgG Purification System was developed to overcome the drawbacks of the commonly used immobilized Protein A and Protein G purification methods. Protein A and Protein G affinity methods have selective binding of IgG species and their subclasses. The procedure is typically labor-intensive and requires harsh elution conditions to disrupt the affinity interaction, and the purified antibody usually requires dialysis or desalting before storage or use in downstream applications. The Melon Gel method eliminates the need for an elution step and uses a mild purification buffer at physiological pH that is free of primary amines and, therefore, compatible with most downstream applications. The spin-column format can be completed in less than 15 minutes, producing purities greater than 80% and recoveries greater than 90% for a variety of IgG species (Table 1).

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Important Product Information

- Melon Gel will not purify chicken IgY. Comparisons of purification characteristics of IgG species with Melon Gel, Protein A and Protein G are listed in Table 1.
- For the spin-column format, 100µL of settled gel has the capacity to purify 100µL of serum. For the gravity-flow column format, the capacity for 1mL of settled gel is 1-2mL of serum. Using insufficient gel will cause contaminants to flow through the Melon Gel with the purified antibody. Using too much gel can result in binding (loss) of the antibody. Serum typically contains ~10-15mg/mL of IgG; however, results vary depending on species and sample preparation. Melon Gel also can be used in a batch format.
- Transferrin might co-purify with IgG when using mouse and rat serum. Transferrin produced in these species and does not react with the gel in the same manner as transferrin from other species. To reduce the presence of transferrin in the flow-through, perform an ammonium sulfate precipitation before purification (see Additional Information Section).
- The buffer provided is optimal for antibody purification from serum but will not remove hemoglobin. If the presence of hemoglobin hinders subsequent use of the antibodies or the sample is significantly hemolyzed, then use 10mM Tris, pH 8.0 in the purification procedure instead of the Purification Buffer or perform an ammonium sulfate precipitation (see Additional Information Section) and dialysis before using the Melon Gel.
- Monoclonal antibodies may be purified from culture supernatant or ascites fluid using this kit. For a complete protocol, see the instructions for Melon Monoclonal IgG Purification Kit (Product No. 45219). Ascites samples require treatment with Ascites Conditioning Reagent (Product No. 45219).

Table 1. Purification characteristics of IgG species using Melon Gel, Protein A and Protein G.

Source	Melon™ Gel	Protein A	Protein G
Human	G	G	G
Mouse	G	G	G
Rabbit	G	G	G
Rat	G	W	M
Goat	G	W	G
Cow	M	W	G
Sheep	M	W	G
Horse	G	W	G
Guinea Pig	G	G	W
Pig	G	G	W
Chicken	N	N	N
Hamster	G	M	M
Donkey	G	M	G

Legend: G = good purification; M = medium purification; W = weak purification; N = does not purify

Material Preparation

Melon Gel Purification Buffer	To make the 100X stock solution, reconstitute the Purification Buffer with 950mL of ultrapure water. Before use, dilute the stock solution 1:100 with ultrapure water and adjust the pH to 6.5-6.7 by adding ~1mL/L of 0.5M sodium hydroxide. For long-term storage, sterile filter the stock solution and store at 4°C. Sodium azide may be added to a final concentration of 0.02%.
Melon Gel Regenerant	Reconstitute the Regenerant by adding 865mL of ultrapure water and mixing for 5 minutes to completely dissolve contents. For long-term storage, sterile filter the solution and store at room temperature. Do not store at 4°C as the solution may precipitate.

Gravity-flow Column Procedure for Antibody Purification

For the gravity-flow column format, 1mL of settled gel has the capacity to purify 1-2mL of serum. Typically, gravity-flow columns can be regenerated three times without significant loss of selectivity. Ammonium sulfate precipitation of the sample followed by dialysis and filtration (0.22µm) allows reuse of the column up to six times.

A. Additional Materials Required

- Serum sample: Dilute serum 1:10 with 1X Melon Gel Purification Buffer.

  Note: To avoid diluting sample, dialyze serum using 1-2 changes of 1X Melon Gel Purification Buffer. Use a volume of dialysis buffer at least 300-fold greater than the volume of the sample. Alternatively, for samples that are ≤ 4mL, perform a buffer exchange using a Zeba™ Desalt Spin Column (see Related Pierce Products Section).
- 1,000µL pipettor and pipette tips, including one large-orifice or cut pipette tip for dispensing gel slurry
- End-over-end rocker or rotator
- Disposable column capable of containing at least 1mL gel bed volume such as the Disposable Polypropylene Columns (Product No. 29922) or the Column Trial Pack (Product No. 29925) that contains two each of three column sizes.

B. Gravity-flow Column Protocol

1. Equilibrate the Melon Gel IgG Purification Support and Purification Buffer to room temperature (~30 minutes).
2. Swirl bottle containing the Melon Gel (do not vortex) to obtain an even suspension. To ensure proper gel slurry dispensing, use a wide orifice or cut pipette tip.
3. Carefully pack a column with the gel support following the instructions provided with the columns. Use a gel-bed volume of 0.5-2.0 times the undiluted serum volume. For example, use 1-4mL of settled gel (5-20mL of 20% gel slurry) per 2mL of undiluted serum.
4. After column stops flowing, add 10 times the gel-bed volume of 1X Melon Gel Purification Buffer and allow it to flow through the column.
5. Add the serum sample. The IgG will begin to emerge after the column void volume has flowed through the column. (The column void volume is ~75% of the gel-bed volume.) Collect 0.5-1mL fractions.

   Note: If the sample was dialyzed or in a volume less than the column void volume, allow sample to enter the gel bed and add 1X Melon Gel Purification Buffer to collect fractions.
6. Measure the absorbance of each fraction at 280nm. Add 1X Melon Gel Purification Buffer and continue to collect fractions until sample approaches baseline absorbance. Use the antibody directly for downstream applications or store it for future use.
7. Regenerate column with 10 times the gel-bed volume of Regenerant followed by 10 times the gel-bed volume of ultrapure water.
8. For storage, wash column with 10 times the gel-bed volume of 1X Melon Gel Purification Buffer. When approximately 1mL of Purification Buffer remains above the gel, place the bottom cap followed by the top cap on the column. Store the column upright at 4°C. 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.

Microcentrifuge Spin-column Procedure for Antibody Purification

Note: For the spin-column format, 100µL of settled gel has the capacity to purify up to 100µL of serum. This spin-column procedure can be scaled up for use in Pierce Centrifuge Columns (e.g., Product No. 89898, 10mL).

C. Additional Materials Required

- Serum Sample: Dilute 10-100µL of serum 1:10 in 1X Melon Gel Purification Buffer. Serum samples exceeding 50µL (i.e., > 500µL upon dilution) must be processed in two batches. **Note:** To avoid diluting sample, perform a buffer exchange using a Zeba Desalt Spin Column (see Related Pierce Products Section).
- Spin columns such as the Pierce Mini-Spin Columns and Accessories (Product No. 69705)
- 200µL and 1,000µL pipettors and pipette tips, including one large-orifice or cut pipette tip for dispensing gel slurry
- Microcentrifuge set to moderate speed (2,000-6,000 × *g*) – using centrifugal forces greater than 6,000 × *g* produces sub-optimal results
- End-over-end rocker or rotator

D. Spin Column Protocol

1. Equilibrate Melon Gel IgG Purification Support and Purification Buffer to room temperature (~30 minutes).
2. Swirl bottle containing the Purification Support (do not vortex) to obtain an even suspension. To ensure proper gel slurry dispensing, use a wide bore or cut pipette tip to dispense 500µL of gel slurry into a Pierce Mini-Spin Column placed in a microcentrifuge tube. Swirl the bottle of gel slurry before pipetting each sample to maintain the gel suspension.
3. 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 × *g*. Using centrifugal forces greater than 6,000 × *g* produces sub-optimal results.
4. 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.

5. Add 100-500µL of diluted or 10-100µL of buffer-exchanged serum to the column. Sample volumes greater than 500µL must be processed in two batches. Do not use gel more than two times without regenerating.
6. Cap column and incubate for 5 minutes at room temperature with end-over-end mixing.
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. Repeat Steps 5-7 for the second batch. Use the antibody directly for downstream applications or store it as desired.
9. Discard or regenerate the used gel support. For gel regeneration, perform the following steps:
   - Add 500µL of Melon Gel Regenerant, mix for 5 minutes, centrifuge and discard flow-through.
   - Add 500µL Purification Buffer, centrifuge and discard flow-through. Repeat this wash step gel five times.
   - Add 500µL Purification Buffer and store at 4°C. The gel can be regenerated three times without significant loss of selectivity.

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
Considerable antibody purified, but no antibody of interest detected	Antibody of interest is at low concentration	Affinity purify the antibody using the specific antigen coupled to an activated affinity support such as AminoLink® Plus Immobilization Kit (see Related Pierce Products)
Non-antibody bands present on stained SDS-polyacrylamide gel	Sample contains salts > 25mM and/or pH is not neutral	Dialyze sample against the Purification Buffer
		Ensure the sample pH is 6.5-7.0
		If using a gravity-flow column that was regenerated, thoroughly wash column to remove all regenerant followed by column equilibration with Purification Buffer
Abundant non-IgG proteins recovered	Centrifugations were performed at forces greater than 6,000 × *g*	Perform all centrifugation steps at 2,000-6,000 × *g*
Purified IgG is colored	Serum sample is hemolyzed	See the Additional Information Section for suggestions for hemolysis elimination

Additional Information

A. Ammonium Sulfate Precipitation[1]

1. Prepare saturated ammonium sulfate solution by dissolving 76.1g ammonium sulfate in 100mL ultrapure water. Alternatively, purchase Saturated Ammonium Sulfate (Product No. 45216).
2. Measure the volume of the serum sample and slowly add an equal volume of the saturated ammonium sulfate solution.
3. Allow sample to precipitate on ice for 2-4 hours or overnight at 4°C.
4. Centrifuge sample at 3,000 × *g* for 20 minutes.
5. Discard the supernatant and dissolve the precipitate in Melon Gel Purification Buffer. Use a volume of buffer equivalent to the original volume of the serum sample.
6. Dialyze sample against three changes of 1X Melon Gel Purification Buffer. Use a dialysis buffer volume at least 300-fold greater than the volume of the sample.

B. Hemolysis Elimination

Hemolysis of serum sample can be reduced or eliminated by performing any of the following procedures.

- Clot blood the same day of collection – do not clot blood overnight
- Collect blood in the presence of an anti-coagulant and centrifuge to remove the red blood cells
- Collect blood with care to prevent hemolysis
- Collect interstitial fluid instead of blood
- Perform an ammonium sulfate precipitation to remove contaminants

C. Information Available from our website

- Tech Tip Protocol: Remove Air Bubbles from Columns
- Tech Tip Protocol: Degas Solutions for use in Affinity Columns
- Tech Tip: Protein Stability and Storage

Related Thermo Scientific Products

45206	**Melon Gel IgG Spin Purification Kit,** 3mL of Melon Gel, buffers and microcentrifuge mini-spin column accessories for purification from small amounts of serum
45214	**Melon Gel Monoclonal IgG Purification Kit,** 200mL Melon Gel and buffers for large-scale purification from culture supernatant, ascites or serum samples
45219	**Ascites Conditioning Reagent,** 5mL, sufficient to treat 125mL ascites before Melon Gel purification
45216	**Saturated Ammonium Sulfate,** 1L
69705	**Pierce Mini-Spin Columns and Accessories,** 25 units
69720	**Pierce Microcentrifuge Tubes,** for use with Product No. 69705
89882	**Zeba Desalt Spin Columns, 0.5mL,** 25 columns, for 30-130µL samples
89889	**Zeba Desalt Spin Columns, 2mL,** 5 columns, for 200-700µL samples
89891	**Zeba Desalt Spin Columns, 5mL,** 5 columns, for 500-2,000µL samples
89893	**Zeba Desalt Spin Columns, 10mL,** 5 columns, for 700-4,000µL samples
66382	**Slide-A-Lyzer® Dialysis Cassette Kit**, 10 dialysis cassettes, each appropriate for 0.5-3.0mL samples
66529	**Slide-A-Lyzer Concentrating Solution**, 500mL
25200-25244	**Precise™ Protein Gels** (see catalog or web site for a complete listing)
44894	**AminoLink Plus Immobilization Kit**
21435	**EZ-Link® Sulfo-NHS-LC-Biotinylation Kit**

Reference

1. Harlow, E. and Lane, D. (1988). Antibodies: A Laboratory Manual. Cold Spring Harbor Laboratory; New York: p. 298-299.

This product ("Product") is warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation") and to be free from defects in material and workmanship. Unless otherwise expressly authorized in writing, Products are supplied for research use only. No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than the original purchaser of the Product ("Buyer").

No other warranties, express or implied, are granted, including without limitation, implied warranties of merchantability, fitness for any particular purpose, or non infringement. Buyer's exclusive remedy for non-conforming Products during the warranty period is limited to replacement of or refund for the non-conforming Product(s).

There is no obligation to replace Products as the result of (i) accident, disaster or event of force majeure, (ii) misuse, fault or negligence of or by Buyer, (iii) use of the Products in a manner for which they were not designed, or (iv) improper storage and handling of the Products.

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

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