INSTRUCTIONS

Melon™ Gel Spin Plate Kit for IgG Screening

45208

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Number	Description
45208	**Melon Gel Spin Plate Kit for IgG Screening**, sufficient materials to process up to 192 samples containing 20-100 µl
	Kit Contents:
	Melon Gel 96-Well Spin Plates, 2 each, contains 100 µl of Melon Gel per well
	Purification Capacity: 1 mg of human IgG/well
	Melon Gel Purification Buffer, 2 × 100 ml
	Wash Plates (white), 2 each
	Collection Plates (blue), 2 each
	Storage: Upon receipt store at 4°C. Product shipped at ambient temperature.

Introduction

The Melon Gel Spin Plate Kit for IgG Screening offers a high-throughput format for quick purification and screening of antibodies. This kit enables purification of up to 96 samples of 20-100 µl in 15 minutes or less. Melon Gel purifies antibodies from serum, cell culture supernatant and ascites fluid by removing non-relevant proteins often present in high abundance, such as albumin and transferrin. The purified antibody flows through the plate wells in a mild buffer at physiological pH suitable for storage and most downstream applications. Cell culture supernatant samples may be applied directly to the Melon Gel without the need for ammonium sulfate precipitation, provided the sample is exchanged into the Melon Gel Purification Buffer. Ascites samples, however, require treatment with the Ascites Conditioning Reagent before purification.

Important Product Information

- The Melon Gel Spin Plates are compatible with centrifugation and positive pressure systems.
- 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.
- Each well contains 100 µl of Melon Gel, which is sufficient to purify/screen IgG from 20-100 µl of serum, cell culture supernatant (containing up to 10% fetal bovine serum) or ascites fluid. Be aware that antibodies from fetal bovine serum (FBS) culture media supplement will co-purify with the antibody of interest.
- Transferrin from various species, including mouse and rat, might be detected in Melon Gel-purified samples. To reduce the presence of transferrin in the flow-through, use a Saturated Ammonium Sulfate Solution (Product No. 45216) for precipitation of contaminants before the purification.
- Prepare ascites samples for purification/screening using the Ascites Conditioning Reagent (Product No.45219). Abundant proteins such as transferrin are detected in unconditioned ascites samples purified with the Melon Gel.
- The buffer provided is optimal for purifying/screening antibodies from non-hemolyzed samples. If the presence of hemoglobin hinders subsequent use of the antibodies or if the samples are significantly hemolyzed, then use 10 mM Tris, pH 8.0 in the procedure instead of the Purification Buffer. Alternatively, use a Saturated Ammonium Sulfate Solution (Product No. 45216) for precipitation of contaminants before the purification. Note that Tris contains primary amines and is not compatible with amine-reactive conjugation chemistries.
- When centrifuging only one Melon Gel Spin Plate, the weight of the plate assembly must be counter-balanced throughout the procedure with a Balance Plate (Product No. 45205) containing an appropriate volume of water.

Table 1. Purification characteristics of IgG species using the 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

Procedure for IgG Purification/Screening from Serum or Cell Culture Supernatant

A. Additional Materials Required

- Plate or orbital shaker
- Variable-speed centrifuge with rotor and carriers capable of handling stacked plates (4.4 cm height) at 1,000 × *g*
- Sample: Dilute 10 µl of sample 1:10 in Melon Gel Purification Buffer or perform buffer exchange of 20-100 µl of sample using the Zeba™ 96-well Desalt Spin Plates (Product No. 89807)

B. IgG Purification/Screening from Serum or Cell Culture Supernatant

1. Equilibrate Melon Gel 96-well Spin Plate and Purification Buffer to room temperature (~30 minutes).
2. Remove the seal from the bottom of the plate and place one plate on top of each wash plate (white).
3. Remove the top seal. Place the assembly into a centrifuge with a 96-well plate-carrier rotor and centrifuge for 1 minute at 1,000 × *g* to remove the storage buffer. Discard the flow-through.
4. Rinse the wash plate with ultrapure water, dry and save for future use.
5. Place the Melon Gel Spin Plate on top of the collection plate (blue), aligning corresponding alphanumeric indices on the plates.
6. Apply the buffer-exchanged sample (20-100 µl) to the center of the gel bed. To expel the entire sample, carefully touch the pipette tips to the gel. For 20 µl samples, apply 20 µl stacker of water or buffer on top of the gel bed after the sample has fully absorbed to ensure optimal sample recovery.
7. Place the plate assembly on a plate or orbital shaker and incubate for 5 minutes with moderate agitation.
8. Centrifuge the plate assembly at 1,000 × *g* for 1 minute to collect the purified antibodies. Discard the used plate.

Procedure for IgG Purification/Screening from Ascites Fluid

Note: Prepare ascites samples using the Ascites Conditioning Reagent (Product No.45219). Abundant proteins such as transferrin are detected in unconditioned ascites samples purified with the Melon Gel.

A. Additional Materials Required

- Ascites Conditioning Reagent (Product No. 45219)
- 500 mM NaCl
- Desalting devices: Zeba Desalt Spin Columns, 0.5 ml (Product No. 89882) or Zeba 96-well Desalt Spin Plates (Product No. 89807)

B. Ascites Conditioning

1. Measure the volume of the sample (20-100 µl) and transfer to a microcentrifuge tube.
2. Measure one half the sample volume of 1X Melon Gel Purification Buffer and add 4 µl of the Ascites Conditioning Reagent for every 100 µl of original sample volume. Pulse vortex for 10 seconds.
3. Slowly add the buffer containing the Ascites Conditioning Reagent to the sample.

   Note: For better antibody recovery and less contaminants in the final product, add the Ascites Conditioning Reagent slowly and with mixing.
4. Allow sample to be conditioned for 10 minutes at room temperature while rocking or rotating. The mixture will be opaque after the conditioning step.
5. Centrifuge sample at 5,000 × *g* for 10 minutes.
6. Remove the supernatant and discard the pellet. Some particulates will remain in the supernatant rather than gathering in the pellet, therefore, take care not to remove particulates with the supernatant.
7. Desalt the sample using Zeba Desalt Spin Columns or Plates pre-equilibrated with Melon Gel Purification Buffer.

   Note: To optimize the desalting capabilities of the Zeba Desalt Spin Columns, load a sample volume less than 10% of the total desalting column volume.
8. Add 10 µl of 500 mM NaCl per 100 µl of sample.

C. IgG Purification/Screening from Ascites

1. Equilibrate Melon Gel 96-well Spin Plate and Purification Buffer to room temperature (~30 minutes).
2. Remove the seal from the bottom of the plate and place the plate on top of the wash plate (white).
3. Remove the top seal. Place the assembly into a centrifuge with a 96-well plate-carrier rotor such that they balance each other and then centrifuge the plates for 1 minute at 1,000 × *g*. Discard the flow-through.
4. Rinse the wash plate three times with ultrapure water. Dry plate and save for future use.
5. Place the purification plate on top of the collection plates (blue), aligning corresponding alphanumeric indices on the plates.
6. Apply the buffer-exchanged sample containing 50 mM NaCl to the center of the gel bed. To expel the entire sample, carefully touch the pipette tips to the gel. For 20 µl samples, apply 20 µl stacker of water or buffer on top of the gel bed after the sample has fully absorbed to ensure sample recovery.
7. Place the plate assembly on a plate or orbital shaker and incubate for 5 minutes with moderate agitation.
8. Centrifuge the plate assembly at 1,000 × *g* for 1 minute to collect the purified antibodies.
9. Discard the plate after use.

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
		If the sample was ascites fluid, make certain that NaCl (see step B8) was added to the sample before it was applied to the Melon Gel
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 a support such as AminoLink® Plus Immobilization Kit (Product No. 44894)
Non-antibody bands present on stained gel	Sample contains salts > 25 mM and/or pH is not neutral	Buffer exchange sample against the Purification Buffer using a desalting column or plate
		Ensure the sample pH is 6.5-7.0
	Ascites sample was not pre-conditioned	Treat ascites fluid with the Ascites Conditioning Reagent (Product No. 45219)
	Centrifugations were performed at forces greater than 1,000 × *g*	Perform all centrifugation steps at 1,000 × *g* (the ascites conditioning protocol is an exception)

Additional Information

Please visit our website for additional information on this product including the following:

- Tech Tip: Protein Stability and Storage

Related Products

45219	**Ascites Conditioning Reagent,** 5 ml, for up to 125 ml of ascites fluid
45216	**Saturated Ammonium Sulfate,** 1 L
15036	**Sealing Tape for 96-well Plates,** 100 each
45206	**Melon Gel IgG Spin Purification Kit,** sufficient reagents to purify up to 3 ml of serum
45212	**Melon Gel IgG Purification Kit,** sufficient reagents to purify up to 50 ml of serum
45214	**Melon Gel Monoclonal IgG Purification Kit**
89807	**Zeba 96-well Desalt Spin Plates,** 2 pack

Reference

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