INSTRUCTIONS

Melon Gel Chromatography Cartridge

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89932 89933

Number	Description
89932	**Melon Gel Chromatography Cartridge,** 2 × 1mL
89933	**Melon Gel Chromatography Cartridge,** 5mL

Purification Capacity: 1-2mL of normal human serum, 1mL of ascites or 5mL of cell culture supernatant per milliliter of resin bed

Note: Ascites samples require treatment with the Ascites Conditioning Reagent (Product No. 45219).

Each Column Pack Contains:

Melon Gel Purification Buffer BupH™ Pack, 1 pack, produces 200mL of 100X buffer

Melon Gel Regenerant BupH Pack, 1 pack, produces 0.5L of 1X solution when reconstituted

Accessory Pack, 1 female Luer-Lok™ Adapter, 1 connector fitting, 1 column plug and 1 or 2 bottom caps

Storage: Upon receipt store at 4-8°C. Product is shipped at ambient temperature. Do not freeze.

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Introduction

The Thermo Scientific™ Melon™ Gel Chromatography Cartridge is a convenient, ready-to-use prepacked chromatography column for the isolation and purification of antibodies. The Melon Gel 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 most downstream applications. Melon Gel overcomes the drawbacks of immobilized Protein A and Protein G purification methods, such as species and subclass selectivity and harsh elution conditions, because Melon Gel purifies most antibody species (Table 1) in a mild buffer that is free of primary amines. With minor sample preparation, antibodies can be purified from a variety of sources including serum, ascites and cell culture supernatant.

The Thermo Scientific™ Pierce™ Cartridges are compatible with major automated liquid-chromatography systems or manual syringe processing (see Table 2 for general properties of the cartridges). The cartridges attach directly to ÄKTA™ or FPLC Systems without additional connectors. An accessory pack, included with each product, readily adapts cartridges for use with Luer-Lok Syringe Fittings or 1/16” tubing. The Melon Gel Cartridges provide fast, easy and reproducible chromatographic separations and can be regenerated for multiple uses.

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

Table 2. Properties of the Melon Gel Cartridges.

Purification Capacity (per mL of resin bed)	1-2mL of normal human serum 1mL of ascites 5mL of cell culture supernatant
Cartridge Dimensions	0.7 × 2.7cm (1mL column); 1.3 × 3.8cm (5mL column)
Particle Size	40-150µm
Void Volume	0.32mL (1mL column); 1.5mL (5mL column)
Max Flow Rate	2mL/min (1mL column); 3mL/min (5mL column)
Recommended Flow Rate	0.5-1.0mL/min (1mL column); 1-2mL/min (5mL column)
pH Limits	2-9.5
Max Operating Pressure	0.3 MPa, 43.5 psi or 3 bar
Cartridge Material	Polypropylene
Frit	Polyethylene, 10µm
Storage Solution	0.02-0.05% Sodium azide
Accessory Pack	Luer-Lok Adapter to 10-32 male Finger-tight 10-32 connector fitting for 1/16" OD tubing Plug for 10-32 coned port Cap 1/16 male

Important Product Information

- This product will work only with the supplied buffers. Additional buffer packs can be purchased separately (see the Related Products section).
- Use high-purity buffers prepared with high-quality water. For best results, degas or filter buffers through a 0.45µm filter.
- 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.
- Overloading the column will cause contaminants to flow through the Melon Gel along with the antibody. Under-loading the resin could result in nonspecific binding of the desired antibody. Serum typically contains 10-15mg/mL of IgG. Results may vary depending on species and sample preparation. For optimal recovery, use 1-2mL of human serum, 1mL of ascites or 5mL of cell culture supernatant per milliliter of resin bed.

  Note: Ascites samples require treatment with the Ascites Conditioning Reagent (Product No. 45219).
- Transferrin from various species, including mouse and rat, may be detected in samples purified with Melon Gel because transferrin produced in these species has different properties and does not react with the resin in the same manner as from other species. To reduce the amount of transferrin in the flow-through, perform an ammonium sulfate precipitation (Additional Information, Procedure B) before purifying.
- The buffer provided is optimal for antibody purification but will not remove hemoglobin from serum samples. If the presence of hemoglobin hinders subsequent use of the antibodies or the sample is significantly hemolyzed, then use 10 mM Tris, pH 8.0 in the purification procedure instead of the Purification Buffer or perform an ammonium sulfate precipitation (see Additional Information Section) and buffer exchange before using the Melon Gel. Note that Tris contains primary amines and is not compatible with amine-reactive conjugation chemistries.
- Prepare ascites samples for purification using the Ascites Conditioning Reagent (Product No. 45219). Abundant proteins, such as transferrin, are detected in unconditioned ascites samples purified with Melon Gel.
- Do not freeze Melon Gel Resin as this will cause irreversible damage to the bead structure.
- Pierce Cartridges may be used singly or connected in series (2-3 columns) to increase capacity. Back pressure will be greater when columns are used in a series than when used as single columns.

Additional Materials Required

- Suitable liquid-chromatography system (LC procedure only) with 1/16" tubing or syringes
- Three-way Luer-Lok Stopcock may be used for syringe processing (optional).
- Additional connectors and fittings are required to attach to the Bio-Rad BioLogic™ System.
- Sample preparation materials: Dialysis or desalting devices and Ascites Conditioning Reagent (see the Sample Preparation Section)

Buffer Preparation

Melon Gel Purification Buffer	To make the 100X stock solution, reconstitute the buffer pack contents with 190mL of ultrapure water and adjust pH to 6.5-6.7 with ~1 mL/L of 0.5M sodium hydroxide. Adjust volume to 200mL with water. . Before use, dilute the stock solution 1:100 with ultrapure water. For long-term storage, sterile filter the stock solution and store at 4ºC. If desired, add sodium azide to a final concentration of 0.02%. Filter through a 0.45µm filter before use in FPLC applications.
Melon Gel Regeneration Buffer	Reconstitute the Melon Gel Regenerant with ultrapure water to a final volume of 500mL. Mix for 5 minutes to completely dissolve contents. Filter through a 0.45µm filter before use in FPLC applications. Store at room temperature. Do not store at 4°C because the solution may precipitate.

Sample Preparation

- **Serum**

Note: For serum, 1mL of resin bed has the capacity to purify 1-2mL of serum.

1. Dilute 0.1-2mL of serum 1:10 in Melon Gel Purification Buffer. Alternatively, perform a buffer exchange using Thermo Scientific Pierce Desalting Cartridges (Product No. 89934, 89935), Zeba Desalting Columns or Slide-A-Lyzer Dialysis Cassettes (see the Related Products Section) to avoid diluting the serum sample.
2. Add 1-5mL of diluted serum or 0.1-2mL of buffer-exchanged serum per milliliter of Melon Gel. Diluted serum samples exceeding 5mL for the 1mL column or 25mL for the 5mL column must be processed in multiple batches.

   Note: Do not apply a cloudy sample to the column. If necessary, filter (0.45µm filter) the sample or centrifuge the solution before applying to the column.
3. Proceed with chromatography using an LC system or syringe.

- **Ascites**

Note: Ascites samples require treatment with the Ascites Conditioning Reagent (Product No. 45219). For ascites, 1mL of Melon Gel has the capacity to purify 1mL of treated sample.

1. Measure the volume of the sample and transfer to a centrifuge tube that can withstand a force of 5,000 × *g*.
2. Place one half of the sample volume of Melon Gel Purification Buffer into a tube and add 40µL of the Ascites Conditioning Reagent for every 1mL of original sample volume. Pulse vortex for 10 seconds.
3. While mixing the sample, slowly add the buffer containing the Ascites Conditioning Reagent. Antibody precipitation results when the solution is added too quickly or when mixing is insufficient.
4. Rock or rotate sample for 10 minutes at room temperature. The mixture appears opaque after conditioning.
5. Centrifuge sample at 5,000 × *g* for 10 minutes. Remove the supernatant and discard the pellet. Be careful not to remove any particulate matter that did not pellet.
6. Dialyze sample with two 1-hour exchanges of Melon Gel Purification Buffer at room temperature or desalt sample using a desalting column pre-equilibrated with Melon Gel Purification Buffer.

   Note: For best results, use a sample volume less than 10% of the total desalting column volume.
7. Add 25µL of Melon Gel Regeneration Buffer per 1mL of conditioned ascites sample and mix well. Proceed with chromatography using an LC system or syringe.

- **Cell Culture Supernatant**

Note: For cell culture supernatants, 1mL of resin bed has the capacity to purify 5mL of sample containing ≤ 10% fetal bovine serum (FBS).

1. Dialyze the supernatant using two 1-hour exchanges of Melon Gel Purification Buffer. Use a volume of dialysis buffer at least 50-fold greater than the sample volume. Alternatively, for samples ≤ 4mL, exchange the buffer using Pierce Desalting Cartridges or Zeba™ Desalting Columns (see the Related Products Section).
2. Proceed with chromatography using an LC system or syringe.

Procedure for Antibody Purification Using a Liquid-Chromatography System

1. Equilibrate the cartridge and all buffers to room temperature. Ensure all solutions are degassed.
2. Prepare the LC system by filling tubing with buffer. Remove top plug from column and carefully snap off bottom end-tab (do not twist). To avoid introducing air into the system, let a few drops of buffer flow from tubing into column top and connect column top to the tubing; allow a few drops to emerge from the column bottom before connecting to the LC inlet port.
3. Equilibrate the resin with 5-10 column volumes of the Melon Gel Purification Buffer at a flow rate of 1mL/minute for the 1mL column or 2mL/minute for the 5mL column.
4. Apply the prepared sample to the column at a flow rate of 0.5mL/min for the 1mL column or 1mL/minute for the 5mL column.
5. Add 2-10 column volumes of Melon Gel Purification Buffer to the cartridge. Purified antibodies flow through and the non-antibody serum proteins bind to the resin. Collect fractions containing the purified antibody.

   Note: The purified antibodies flow through in mild buffer suitable for storage and many downstream applications and may be used directly in SDS-PAGE.
6. Typically, the resin may be regenerated multiple times without significant loss of selectivity by washing with five column volumes of Melon Gel Regeneration Buffer or 0.5N NaOH. Equilibrate resin with Melon Gel Purification Buffer (i.e., 10 column volumes or until conductivity approaches the value of the purification buffer) and store at 4°C. If storing for > 1 week, add a final concentration of 0.02% sodium azide to the buffer used to wash the resin.

Procedure for Antibody Purification Using a Syringe

Note: The void volumes are 0.320mL for the 1mL columns and 1.5mL for the 5mL columns.

1. Equilibrate the cartridge and all buffers to room temperature. Ensure all solutions are degassed.
2. Fill a syringe with 5-10 column volumes of 1X Melon Gel Purification Buffer.
3. Attach the syringe to the Luer-Lok Adapter included in the accessory pack. Remove top plug from column and carefully snap off bottom end-tab. To avoid introducing air into the system, allow a few drops to emerge from the adapter end before connecting syringe to column top. Securely tighten the connection.
4. Equilibrate the Melon Gel Resin with 5-10 column volumes of purification buffer at a flow rate of ~1mL/minute for the 1mL column or ~2mL/minute for 5mL column. Remove syringe from the Luer-Lok Adapter.
5. Fill an appropriate sized syringe with the prepared sample and connect to the Luer-Lok Adapter. For maximum binding, apply the sample to the column at a flow rate of 0.5mL/minute for the 1mL column or 1mL/minute for the 5mL column.
6. Change syringe and apply approximately 2-10 column volumes of Melon Gel Purification Buffer. Purified antibodies flow through and the non-antibody serum proteins bind to the resin. Collect fractions containing the purified antibody.

   Note: The purified antibodies flow through in mild buffer suitable for storage and most downstream applications and can be used directly for SDS-PAGE.
7. Typically, Melon Gel may be regenerated multiple times without significant loss of selectivity by washing with five column volumes of Melon Gel Regeneration Buffer or 0.5N NaOH. Equilibrate resin with Melon Gel Purification Buffer (i.e., 10 column volumes or until conductivity approaches the value of the purification buffer) and store at 4°C. If storing for > 1 week, add a final concentration of 0.02% sodium azide to the buffer used for washing. To prevent cross-contamination, reuse the columns only with the same type of antibodies.

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 did not flow through	Ensure the sample pH is 6.5-7.0
		Add 25µL of Melon Gel Regeneration Buffer per 1mL of the conditioned ascites as indicated
		If serum-free media was used, decrease the amount of Melon Gel
	The FBS proteins are significantly more abundant than the antibody of interest	Enrich for the antibody by concentrating the cell culture supernatant using a device with a ≥ 75K molecular-weight cutoff
Considerable antibody purified, but no antibody of interest detected	Antibody of interest is at low concentration	Affinity-purify antibody using the specific antigen coupled to a support (e.g., AminoLink™ Plus Kit, Product No. 44894)
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 or if volume is < 4mL, perform a buffer exchange using a desalting column
		Ensure the sample pH is 6.5-7.0
		If using regenerated Melon Gel, thoroughly wash resin to remove all Regenerant then equilibrate column with water and Purification Buffer
	Ascites sample was not properly prepared	Treat ascites with Ascites Conditioning Reagent (Product No. 45219)
		Dialyze sample against the Purification Buffer, or if volume is < 4mL, perform a buffer exchange using a desalting column
		When using the Zeba Desalt Column for removing the conditioning reagent, add ≤ 10% of the column volume

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 on Our Website

- Tech Tip Protocol: Remove Air Bubbles from Columns to Restore Flow Rate
- Tech Tip Protocol: Degas Buffers for Use in Affinity and Gel Filtration Columns
- Tech Tip: Protein Stability and Storage

Related Products

45219	**Ascites Conditioning Reagent,** 5mL, sufficient to treat 125mL ascites before Melon Gel Purification
45216	**Pierce™ Saturated Ammonium Sulfate Solution,** 1L
89972	**Melon Gel Purification Buffer,** makes 1L of 10X buffer when reconstituted
89973	**Melon Gel Regenerant,** makes 0.5L of 1X solution when reconstituted
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
89935	**Zeba Desalting Chromatography Cartridge, 7K MWCO** 5 × 5mL
89882	**Zeba Spin Desalting Columns, 7K MWCO, 0.5mL,** 25 columns
89889	**Zeba Spin Desalting Columns, 7K MWCO, 2mL,** 5 columns
89891	**Zeba Spin Desalting Columns, 7K MWCO, 5mL,** 5 columns
89893	**Zeba Spin Desalting Columns, 7K MWCO, 10mL,** 5 columns
66382	**Slide-A-Lyzer Dialysis Cassette Kit**, 10 dialysis cassettes, each appropriate for 0.5-3.0mL samples
89806	**Protein Stabilizing Cocktail** (4X), 10mL
XP04200BOX	**Novex™ Tris-Glycine protein gels** (see thermofisher.com/proteingels for a complete listing)

Reference

1. Harlow, E. and Lane, D. (1988). Antibodies: A Laboratory Manual. Cold Spring Harbor Laboratory; New York: p. 298-9.