Agilent Multiple Affinity Removal Spin Cartridges for the Depletion of High-Abundant Proteins from Mouse Proteomic Samples

Instructions

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General Information

Introduction

The Agilent Multiple Affinity Removal System comprises a family of immunodepletion products based on antibody-antigen interactions and optimized buffers for sample loading, washing, eluting, and regenerating. This spin cartridge is specifically designed to remove three high-abundant proteins from mouse biological fluids such as serum and plasma. This technology enables removal of albumin, IgG, and transferrin with a single device. The targeted high-abundant proteins are simultaneously removed by the immobilized antibodies when crude biological samples are passed through the cartridge. Selective immunodepletion provides an enriched pool of low-abundant proteins for downstream proteomics analysis. Removal of high-abundant proteins enables improved resolution and dynamic range for one-dimensional gel electrophoresis (1DGE), two-dimensional gel electrophoresis (2DGE), and liquid chromatography/mass spectrometry (LC/MS). The collected flow-through fractions may need to be concentrated dependent upon the downstream applications.

Multiple Affinity Removal System

The Agilent Multiple Affinity Removal System is specially designed to allow for close study of low-abundant proteins present in flow-through fractions (see Figure 1).

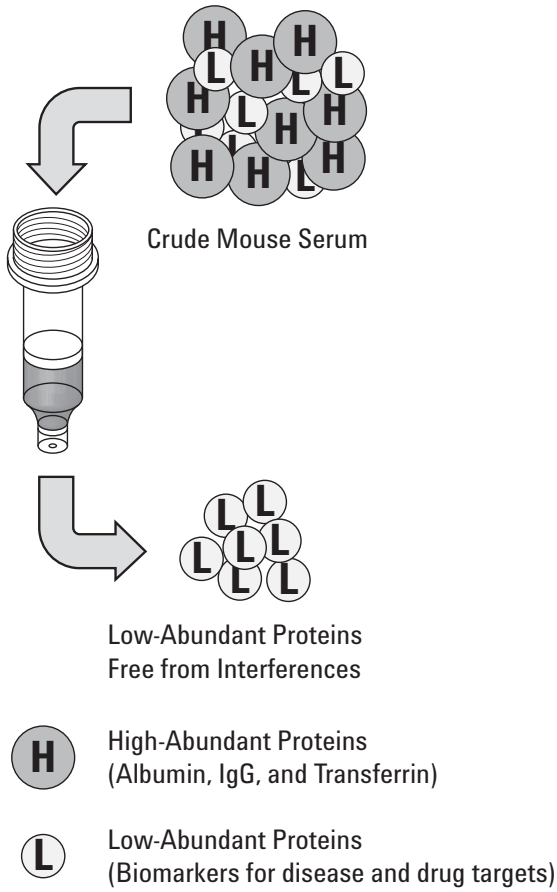

Figure 1 The Multiple Affinity Removal System.

Product Description

The Multiple Affinity Removal Spin Cartridge and its accessories are shown in Table 1.

Table 1 Multiple Affinity Removal Spin Cartridge and Starter Reagent Kit

Product no.	Product name	Product description
5188-5289	0.45-mL affinity, spin cartridge, 1 each	Removes mouse albumin, IgG, and transferrin
5185-5987	Buffer A, 1 L	Ready-to-use, optimized buffer for loading, washing, and equilibrating cartridge
5185-5988	Buffer B, 1 L	Ready-to-use, optimized buffer for elution of bound proteins from cartridge
5185-5990	Spin filters 0.22 µm, 1 pack of 25	For sample cleanup before loading cartridge
5185-5991	Protein spin concentrators, 5k Da MWCO, 4 mL, 1 pack of 25	For concentrating flow-through fractions
5188-5249	Luer-Lock adapters, pack of 2	Allows attachment of Luer-Lock syringes to spin cartridge

Product no.	Product name	Product description
5188-5250	5-mL plastic Luer-Lock syringes, 1 pack of 2	For washing, eluting, and re-equilibrating buffers through spin cartridge
5188-5251	1.5-mL Screw-top microtube, 1 pack of 100	Eppendorf-style tubes used for collecting fractions from spin cartridge
5188-5252	Spin cartridge screw caps and plugs, 1 pack of 6 each	Extra caps and plugs for sealing the top and bottom of affinity spin cartridges
5188-5253	Teflon Luer-Lock needles, 1 pack of 10	For transferring solutions with Luer-Lock syringes
5188-5254	**Starter Reagent Kit for Spin Cartridges*** Buffer A: 1 L Buffer B: 1 L Spin filters 0.22 µm: 2 packs of 25 Protein concentrators: 1 pack of 25 Luer-Lock adapters: 1 pack of 2 5-mL Plastic Luer-Lock syringes: 1 pack of 2 1.5-mL Microtubes: 6 packs of 100 Spin cartridge extra caps and plugs, 1 pack of 6 each Teflon Luer-Lock needles, 1 pack of 10	

* Under normal conditions, the kit should last for approximately 200 spin cartridge uses.

NOTE For higher capacity Multiple Affinity Removal Devices, and if automated immunodepletion is needed, refer to Multiple Affinity Removal Columns (part number 5188-5217, 4.6 × 50 mm and 5188-5218, 4.6 × 100 mm columns for mouse serum) for use with HPLC instrumentation.

Full Protocol for 0.45-mL Multiple Affinity Removal Spin Cartridge

(Cartridge capacity: 25-30 µL mouse serum*)

NOTE During use, never let the spin cartridge frits or resin bed run dry. If this happens, see "Recommendations" section for a cartridge rewetting procedure.

* Consult spin cartridge certificate of analysis to verify your cartridge capacity. Capacity was determined using pooled Swiss Webster mouse serum. Mouse serum protein concentrations can vary for other strains and your sample size should be adjusted accordingly.

Additional Materials Required

- Microcentrifuge with adjustable centrifugal force (capable of spinning at 100 × g) and timer such as the Eppendorf Model 5415D
- 50-mL vessels (for example, polypropylene tubes) to hold small quantities of Buffers A and B during procedure
- Adjustable pipettes for delivering up to 400-µL aliquots
- Transfer pipettes
- 1.5-mL screw-top Microtubes/Eppendorf-style tubes (collection tubes, part number 5188-5251)
- Luer-Lock adapters (part number 5188-5249)

(continued on next page)

- 5-mL plastic Luer-Lock syringes (part number 5188-5250)
- Buffer A, 1 L (part number 5185-5987)
- Buffer B, 1 L (part number 5185-5988)

Material Preparation

- Fill two 50-mL vessels with appropriate amounts of Buffers A and B to use throughout the procedure for your specific number of samples you wish to process (approximately 5-mL Buffer A and 2-mL Buffer B per each 25-30 µL serum sample).
- Label two 5-mL Luer-Lock syringes with “A” and “B” for using later in the procedure during the cartridge elution and re-equilibration steps.

NOTE Remove Agilent Multiple Affinity Removal Spin Cartridge from refrigerator and allow cartridge to equilibrate to room temperature before use.

Procedure

1. **Dilute and filter sample.** Prepare your sample by diluting 25-30 µL of mouse serum* (consult cartridge certificate for actual serum capacity) with Buffer A† to a final volume of 200 µL. For example: if the recommended cartridge loading capacity on the certificate is 30 µL of serum, dilute 30 µL of serum with 170-µL Buffer A to a final volume of 200 µL.

   If you plan to perform several successive runs on the cartridge, increase amount of diluted sample accordingly.

   Filter diluted samples through a 0.22-µm spin filter (part number 5185-5990) to prevent clogging of spin cartridge frits.

2. **Prepare spin cartridge.** Remove cartridge cap and plug. Attach Luer-Lock adapter to spin cartridge, draw 4 mL of Buffer A into syringe and then attach it to Luer-Lock on spin cartridge. Dispense Buffer A through spin cartridge to prepare resin and to remove any trapped air bubbles. With a transfer pipette, remove any excess Buffer A from top of the spin cartridge.

* Protocol may be applied to other mouse biological fluids like plasma.

† Addition of protease inhibitors in Buffer A for sample dilution helps prevent protein degradation.

3 **Apply sample.** Remove the Luer-Lock adapter and place spin cartridge in a screw-top collection tube and label it “Flow-through 1” or “F1”. Add 200 µL of diluted serum sample to top of resin bed and centrifuge for 1.5 minutes at 100 × g (or lowest possible setting on centrifuge - See note below). Cap the spin cartridge loosely or leave open during centrifugation so the sample is able to flow. Collect the flow-through fraction in the collection tube F1. The resin bed and frits should remain moist, not dry after centrifugation.

NOTE If centrifuge cannot be programmed to 100 × g, then cartridge capacity may be different for use; the optimum results for depletion are obtained when the flow rate is controlled to 0.2 mL/min.

4 **Wash and collect flow-through fraction F1.** Add 400 µL of Buffer A to the top of the resin bed and centrifuge for 2.5 minutes at 100 × g. Collect the flow-through fraction into the same F1 collection tube.

5 **Wash and collect additional flow-through fraction F2.** Place spin cartridge into a new collection tube labeled "Flow-through 2" or "F2". Add 400 µL of Buffer A to the top of the resin bed and centrifuge for 2.5 minutes at 100 × g. Collect the flow-through fraction into the F2 collection tube.

6 **Prepare for elution.** Remove the spin cartridge from the F2 collection tube and attach Luer-Lock adapter to the cartridge top.

7 **Elute bound fraction.** Fill a 5-mL plastic Luer-Lock syringe (labeled "B") with 2 mL of Buffer B and attach to the spin cartridge via the Luer-Lock adapter. Elute bound high-abundant proteins into a new collection tube by slowly pushing Buffer B through the spin cartridge. Save the bound fraction for analysis if desired, or discard it. Do not push air through the spin cartridge and do not allow the resin bed or frits to run dry.

NOTE If the meniscus of Buffer B does not reach the top frit after depressing the syringe plunger completely, remove syringe and draw plunger back to the 1-mL mark with air and re-attach to the cartridge. Use the air in the syringe as positive pressure to push Buffer B through until the meniscus of Buffer B reaches the top frit.

8 **Re-equilibrate.** Remove Buffer B syringe and attach a 5-mL syringe (labeled "A") containing 4 mL of Buffer A to the spin cartridge. Re-equilibrate spin cartridge by slowly pushing Buffer A through the resin bed. Do not allow the resin bed or frits to run dry by leaving a small aliquot of buffer on the top of the frit. The spin cartridge is ready for the next sample. For storage, leave the resin bed wet with Buffer A, and leave a layer of Buffer A above the top frit. Recap both ends of the spin cartridge tightly.

NOTE Be careful when placing plug in lower end that it does not displace or puncture the lower frit. Store spin cartridge in Buffer A in a refrigerator at 2-8 °C (35-46 °F). DO NOT FREEZE THE SPIN CARTRIDGE.

9 Analyze. Analyze separately or combine flow-through fractions F1 and F2 containing the low-abundant proteins. For 1D-SDS-PAGE, an aliquot of the flow-through fraction may be used directly. For IEF, 2DGE, and MS analysis of the flow-through fraction, it is necessary to buffer exchange/desalt to an appropriate buffer. The 5KDa MWCO spin concentrators (part number 5185-5991) may be used for buffer exchange and concentration. Alternatively, for automated desalting and concentration, the Agilent mRP-C18 column (part number 5188-5231) may be used according to published methods (Agilent Technologies, publication 5989-2506EN). For 1D-SDS-PAGE, the flow-through fraction may be used directly. For IEF, 2DGE, and MS analysis of the flow-through fraction, buffer exchange to an appropriate buffer is necessary.

NOTE For maximum recovery of the flow-through protein combine and concentrate fractions F1 and F2.

Recommendations

- **Sample dilution**
  It is not recommended to load crude serum directly onto the spin cartridge. Follow instructions for serum dilution. Addition of protease inhibitors in Buffer A for sample dilution helps prevent protein degradation.
- **Sample cleanup**
  Mouse serum may contain particulate materials that can be removed by a quick spin using a 0.22-µm spin filter (part number 5185-5990).
- **Sample concentration**
  For further down-stream proteomic analysis (SDS-PAGE or LC/MS), combine flow-through fractions F1 and F2 and concentrate the samples. Spin concentrators with 5 KDa MWCO (part number 5185-5991) can be used to concentrate proteins before analysis.
- **Bound fraction analysis**
  If you prefer to analyze the bound fraction, perform a buffer exchange to phosphate-buffered saline (PBS) or to another buffer compatible with your analysis. Buffer B contains compounds that may interfere with some protein assays.

- **Cartridge rewetting**
  If the resin bed becomes dry during cartridge centrifugation or syringe elution, attach a syringe with Buffer A to the spin cartridge via the Luer-Lock adapter and re-equilibrate the cartridge by passing Buffer A through it. This should not affect the spin cartridge performance.
- **Spin cartridge performance**
  Multiple Affinity Removal Spin Cartridges should perform reproducibly for at least 200 runs under proper conditions. Buffers A and B are optimized to support cartridge performance and longevity. We cannot guarantee spin cartridge performance if other buffers are used. Do not expose cartridges to organic solvents (like alcohols, acetonitrile, etc.), strong oxidizers, acids, or reducing agents, and other protein denaturing agents.
- **Cartridge storage**
  Always store Multiple Affinity Removal Spin Cartridges after equilibrating with Buffer A in a refrigerator at 2-8 °C (35-46 °F) when not in use to minimize loss in cartridge capacity.
- **Lyophylization of flow-through fractions**
  Buffer exchange to a volatile buffer (for example, ammonium bicarbonate) is recommended prior to lyophylization due to high salt concentration in Buffer A.

Troubleshooting

Problem	Cause	Solution
No flow	The spin cartridge may be capped too tightly during centrifugation.	Remove or loosen cap during centrifugation.
	Bubble under resin or frits.	Rewet with Buffer A (see “Recommendations - Cartridge rewetting”).
Incomplete flow	See No flow.	--
	Centrifuge parameters need to be adjusted	Adjust centrifuge force and time to achieve ≤0.2-mL/min flow rates through spin cartridge during step 3 through step 5 of procedure.
No proteins in bound fraction	Buffers A and B reversed	Re-equilibrate spin cartridge with Buffer A (step 8) and start over with correct buffer sequence.

Problem	Cause	Solution
Break-through of high-abundant proteins in flow-through fractions F1 and F2	Exceeding cartridge serum capacity	Reduce serum load per sample.
	Serum protein levels may be unusually high	Reduce serum load per sample.
	Flow rate through spin cartridge during sample loading too high	Reduce centrifuga-tion force and/or time during sample loading to not exceed 0.2 mL/min flow rate.

Cartridge Specifications

Parameter	Description
Part number 5188-5289	0.45 mL Multiple Affinity Removal Spin Cartridge 1 each
Body material	Polypropylene
Frit materials	Polyethylene with 10-µm pore size
Cartridge capacity*	25-30 µL mouse serum
Recommended centrifugal force	100 × g
Operating temperature	18-25 °C
Cartridge packing material	Antibody-modified resin
Immobilized ligands	Affinity-purified polyclonal antibodies to mouse albumin, IgG, and transferrin
Shipping solution	Buffer A with 0.02% sodium azide
Shipping temperature	2-8 °C (35-46 °F)
Storage temperature	2-8 °C (35-46 °F)

* For exact cartridge capacity, consult your cartridge certificate of analysis.

Safety Issues

When preparing biological samples using Multiple Affinity Removal Spin Cartridges, follow general guidelines for handling biological materials and wear protective eyewear and gloves.

Related Agilent Products

Part number	Description
5188-5230	**Multiple Affinity Removal Spin Cartridge Hu-6, 0.45 mL**, spin cartridge that depletes six high-abundant proteins (albumin, IgG, IgA, transferrin, haptoglobin, and antitrypsin) from human serum samples, 7-10 µL serum capacity per use
5185-5984	**Multiple Affinity Removal Column Hu-6, 4.6 × 50 mm**, LC column that depletes six high-abundant proteins from human serum samples, 15-20 µL serum capacity per injection
5185-5985	**Multiple Affinity Removal Column Hu-6, 4.6 × 100 mm**, LC column with 30-40 µL human serum capacity per injection
5188-5217	**Multiple Affinity Removal Column Ms-3, 4.6 × 50 mm**, LC column that depletes three high-abundant proteins from mouse serum samples, 37-50 µL serum capacity per injection
5188-5218	**Multiple Affinity Removal Column Ms-3, 4.6 × 100 mm**, LC column with 75-100 µL mouse serum capacity per injection
5185-5986	**Multiple Affinity Removal System Reagent Kit**, starter reagent kit containing buffers, spin filters, and spin concentrators for use with Multiple Affinity Removal LC Columns

For more information or technical assistance, please call toll free: 1-800-227-9770 or visit our website at: www.agilent.com/chem/bioreagents.

CAUTION

Do not expose cartridges to organic solvents (like alcohols, acetonitrile, etc.), strong oxidizers, acids, or reducing agents, and other protein denaturing agents.

WARNING

For **RESEARCH USE ONLY** - this product is **NOT TO BE USED AS AN IN-VITRO DIAGNOSTIC.**

Storage: Store the affinity cartridge at 2-8 °C (35-46 °F) upon receiving and when not in use. **DO NOT FREEZE THE CARTRIDGE!** Store cartridge wetted with Buffer A and with the end-caps tightly sealed.

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