



Multiple Affinity Removal Spin Cartridge Human-6HC, 0.45 ml

Part No. 5188-5341

**For depletion of six high-abundance proteins
from human samples**

Instructions

Version A1, December 2018

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procedures.**



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Notices

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Agilent Technologies, Inc.
5301 Stevens Creek Blvd
Santa Clara, CA 95051 USA

Technical Support

For US and Canada

Call (800) 227-9770 (option 3,4,4)

Or send an e-mail to:
techservices@agilent.com

For Europe, Middle East, Africa, and India

Call 00800 345 600 (toll free) or
+49 69 8679 7730

Or send an e-mail to:
genomics_tech_europe@agilent.com

For all other regions

Agilent's world-wide sales and support center telephone numbers can be obtained at www.agilent.com under Contact Us.

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genomics@agilent.com

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In this Guide...

This document describes how to use the Multiple Affinity Removal Spin Cartridge Human-6HC, 0.45 ml to chromatographically remove six interfering high-abundance proteins from human biological samples prior to LC/MS or electrophoretic analysis of the samples.

1 Before You Begin

This chapter contains information (such as required reagents and equipment) that you should read and understand before you start an experiment.

2 Instructions

This chapter describes the protocol for chromatographic removal of the targeted proteins from human samples such as serum and includes troubleshooting information.

3 Reference

This chapter contains reference information including cartridge specifications and a list of related products.

What's New in Version A1

- Updates to description of needles included with reagent kits (see [Table 1](#) on page 7, [Table 6](#) on page 22)
- Correction to part numbers for spin cartridge Starter Reagent Kit, p/n 5188-5254, syringes, p/n 5188-5250, and mRP-C18 desalting column, p/n 5188-5231 (see [Table 1](#) on page 7, [Table 6](#) on page 22)
- Updates to Technical Support contact information (see [page 2](#))

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1 Before You Begin

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Make sure you read and understand the information in this chapter and have the necessary equipment and reagents listed before you start an experiment.



Safety Considerations

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

Materials Required

Ordering information for the Multiple Affinity Removal Spin Cartridge and additional materials used in the protocol is provided in [Table 1](#) (materials from Agilent) and [Table 2](#) (materials from external suppliers.)

NOTE

For higher capacity Multiple Affinity Removal Devices, and if automated immunodepletion is needed, refer to the Human-6HC Multiple Affinity Removal Columns for use with HPLC instrumentation. See [Table 5](#) on page 21 for ordering details and see www.agilent.com for more information.

Table 1 Agilent Multiple Affinity Removal Spin Cartridge and Accessories

Agilent Part number	Product name	Description
5188-5341	Multiple Affinity Removal Spin Cartridge Human-6HC, 0.45 ml, 1 each	Removes albumin, IgG, IgA, transferrin, haptoglobin and antitrypsin from human biological samples. Includes spin cartridge, 2 Luer-Lok adapters, 1 screw cap, and 1 plug
5185-5987	Buffer A, 1 L	Ready-to-use, optimized buffer for loading, washing, and equilibrating spin cartridge
5185-5988	Buffer B, 1 L	Ready-to-use, optimized buffer for elution of bound proteins from spin cartridge
5185-5990	Spin filters, 0.22 µm, 1 pack of 25	For sample cleanup before loading spin cartridge
5185-5991	Concentrators, 5 kDa MWCO, 1 pack of 25	For concentrating flow-through fractions
5188-5249	Luer-Lok adapters, pack of 2	Allows attachment of Luer-Lok syringes to spin cartridge
5188-5250	5-mL plastic Luer-Lok syringes, 1 pack of 2	For washing, eluting, and re-equilibrating buffers through spin cartridge
5188-5253	Needles, PTFE, Luer-Lock (1 pack of 10)	For transferring solutions with Luer-Lock syringes

Table 1 Agilent Multiple Affinity Removal Spin Cartridge and Accessories

Agilent Part number	Product name	Description
5188-5251	1.5-mL screwtop microtubes, 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
5188-5254	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-Lok adapters: 1 pack of 2 5-mL plastic Luer-Lok syringes: 1 pack of 2 1.5-mL microtubes: 6 packs of 100 Spin cartridge extra caps and plugs, 1 pack of 6 each Needles, PTFE, Luer-Lock (1 pack of 10)

Table 2 Additional materials required from external suppliers

Material	Supplier
Microcentrifuge with adjustable centrifugal force (capable of spinning at 100 × g) and timer	Eppendorf Model 5415D or equivalent
50-mL polypropylene tubes, or similar vessels to hold small quantities of Buffers A and B during procedure	General laboratory supplier
Adjustable pipettes for delivering up to 400 µL	General laboratory supplier
Transfer pipettes	General laboratory supplier

Storage Conditions

Upon receipt and when not in use, store the spin cartridge at 2°C to 8°C (35°F to 46°F). Store wetted with Buffer A and with the end-caps tightly sealed. **Do not freeze the spin cartridge.**

Overview

The Agilent Multiple Affinity Removal System comprises a family of immunodepletion products based on affinity interactions and optimized buffers for sample loading, washing, eluting, and regenerating. This high-capacity spin cartridge is specifically designed to remove six high-abundance proteins from human biological fluids such as serum, plasma, and cerebrospinal fluid (CSF). This technology enables removal of albumin, IgG, IgA, transferrin, haptoglobin, and antitrypsin with a single device. The targeted high-abundance proteins are simultaneously removed when crude biological samples are passed through the cartridge. Selective immunodepletion provides an enriched pool of low-abundance proteins for downstream proteomics analysis, as depicted in [Figure 1](#) on page 10.

Specific removal of the six high-abundance proteins depletes approximately 85–90% of total protein mass from human serum, facilitating study of the low-abundance proteins in the flow-through fractions. Removal of high-abundance 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.

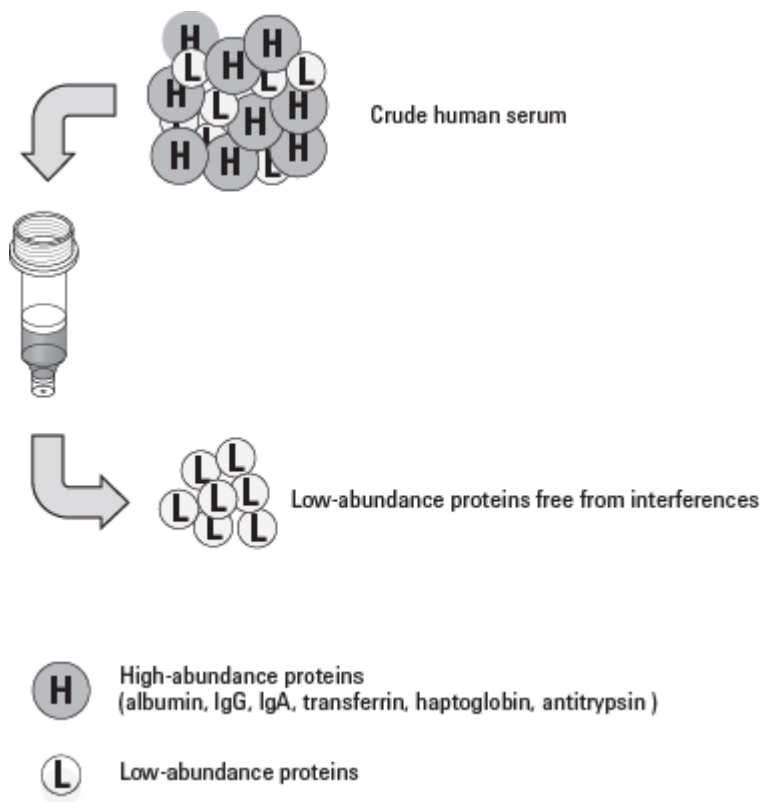
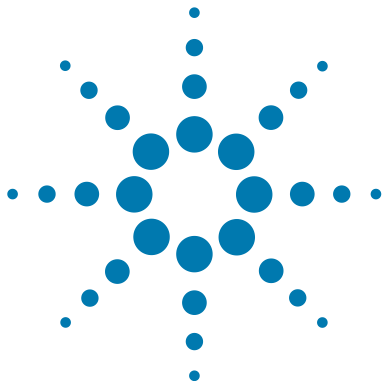


Figure 1 The Multiple Affinity Removal System.



2 Instructions

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Protocol for 0.45-mL spin cartridge

Step 1. Prepare the materials

NOTE

During use, never let the spin cartridge frits or resin bed run dry. If this happens, see “Recommendations” on page 16 for a cartridge rewetting procedure.

- 1 Remove the Multiple Affinity Removal Spin Cartridge from cold storage and allow cartridge to equilibrate to room temperature before use.
- 2 Fill two 50-mL vessels with appropriate amounts of Buffers A and B, according to the number of samples being processed. The affinity removal protocol requires approximately 5 mL Buffer A and 2 mL Buffer B for each 14–16 μ L serum sample.
- 3 Label two 5-mL Luer-Lock syringes with “A” (for use on page 13 and page 14) and “B” (for use on page 14).

Step 2. Prepare the sample

Before you begin, consult the Certificate of Analysis for your cartridge to verify the cartridge capacity. Instructions below are based on a cartridge serum capacity of 14–16 μ L.

- 1 Dilute the 14 to 16- μ L serum sample with Buffer A to a final volume of 200 μ L.

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

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

NOTE

The protocol may be applied to other human biological fluids like plasma and CSF with necessary adjustments in sample volume based on albumin concentration.

- 2 To prevent clogging of spin cartridge frits, remove particulates with a 0.22 μ m spin filter, spinning for 1 min at 16,000 $\times g$.

Step 3. Prepare the spin cartridge

- 1 Remove cartridge cap and plug, retaining both for later cartridge storage.
- 2 Attach Luer-Lok adapter to spin cartridge.
- 3 Draw 4 mL of Buffer A into the syringe labeled “A” and then attach the syringe to the Luer-Lok adapter on the spin cartridge.
- 4 Dispense Buffer A **slowly** 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.

NOTE

To avoid displacing cartridge frits, never apply negative pressure (e.g. by pulling up on the syringe plunger) or excessive positive pressure while dispensing solutions onto the cartridge.

If you experience high backpressure while loading any solution onto the cartridge, it is important to continue loading the solution slowly and to avoid applying extra pressure. See “[Troubleshooting](#)” on page 18 for additional suggestions.

Step 4. Load and run the cartridge

- 1 Remove the Luer-Lok adapter and place spin cartridge in a screw-top collection tube labeled F1 (for flow-through 1).
- 2 Add 200 μ L of diluted serum sample to top of resin bed.
- 3 Centrifuge for 1.5 minutes at $100 \times g$ (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 $100 \times g$, then cartridge capacity may be different for use; optimum depletion results are obtained when the flow rate is controlled to 0.2 mL/min.

- 4 Wash the cartridge with Buffer A and collect additional F1 flow-through fraction. To do this step, add 400 μ L of Buffer A to top of resin bed and centrifuge for 2.5 minutes at $100 \times g$. Collect the flow-through into the same F1 collection tube used in [step 3](#).

- 5 Do second wash with Buffer A and collect F2 flow-through fraction. To do this step, transfer spin cartridge to a fresh screw-top collection tube labeled F2, and add 400 μL of Buffer A to top of resin bed. Centrifuge for 2.5 minutes at $100 \times g$, collecting the flow-through into the F2 collection tube.
- 6 Remove the spin cartridge from the F2 collection tube and attach a Luer-Lok adapter to the cartridge top.
- 7 Elute the bound fraction from the cartridge with Buffer B. To do this step, fill the 5-mL Luer-Lok syringe labeled “B” with 2 mL of Buffer B and attach to the spin cartridge via the Luer-Lock adapter. Elute the bound proteins into a fresh 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 reattach 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 the cartridge with Buffer A. To do this step, fill the 5-mL syringe labeled “A” with 4 mL of Buffer A. Remove the Buffer B syringe from the cartridge and attach the Buffer A syringe. Slowly push 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.
- 9 After equilibration with Buffer A, the spin cartridge is ready for processing the next sample.

If the spin cartridge will be stored before processing the next sample, leave the resin bed wetted with Buffer A, and leave a layer of Buffer A above the top frit. Re-seal both ends of the spin cartridge tightly, taking care not to displace or puncture the lower frit while placing plug in the lower end. Store the cartridge with ends capped at 2°C to 8°C (35°F to 46°F). **Do not freeze the cartridge.**

Step 5. Analyze the flow-through fraction

- 1 For maximum recovery of the low-abundance proteins, combine flow-through fractions F1 and F2. Alternatively, fractions F1 and F2 may be analyzed separately.
- 2 Analyze the flow-through fraction(s), containing the low-abundance proteins, to verify removal of the targeted high-abundance proteins using the guidelines below:
 - For 1D-SDS-PAGE, an aliquot of the flow-through fraction may be used directly.
 - For IEF, 2D-GE, and MS analysis of the flow-through fraction, it is necessary to do buffer exchange or desalt to an appropriate buffer. The 5 kDa MWCO spin concentrators (part number 5185-5991) may be used for buffer exchange and concentration. Alternatively, the Agilent mRP-C18 column (part number 5188-5231) may be used for automated desalting and concentration.

Recommendations

- **Sample dilution using Buffer A**
Do not load crude serum or other biological samples directly onto the cartridge. Follow instructions for serum dilution with Buffer A on [page 12](#).
- **Preventing protein degradation**
Addition of protease inhibitors to Buffer A for sample dilution helps prevent protein degradation.
- **Sample cleanup**
Human serum may contain particulate materials that can be removed by a quick spin using a 0.22- μ m spin filter.
- **Variation in cartridge capacity for different samples**
Concentrations of the proteins targeted for depletion can vary among individual serum samples and in different types of biological samples. Thus cartridge capacity for samples may differ and you may need to adjust the loading volume for a particular sample.
- **Spin Cartridge performance**
Agilent Multiple Affinity Removal Spin Cartridges should perform reproducibly for greater than 200 runs when handled using the recommended procedures. Buffers A and B are optimized to support cartridge performance and longevity. We cannot guarantee cartridge performance if other buffers are used.

Do not expose cartridges to organic solvents (like alcohols, acetonitrile, etc.), strong oxidizers, acids, reducing agents, or other protein-denaturing agents.
- **Cartridge rewetting**
If the resin bed becomes dry during cartridge centrifugation or syringe elution, attach a syringe with Buffer A to the spin cartridge using a Luer-Lok adapter and re-equilibrate the cartridge by passing Buffer A through it. This should not affect the spin cartridge performance.
- **Spin Cartridge storage**
To minimize loss in capacity, equilibrate the cartridge with Buffer A, cap the ends and store at 2°C to 8°C (35°F to 46°F). **Do not freeze the cartridge.**

- **Analysis of flow-through fractions**
Buffer exchange to an appropriate buffer is recommended for high salt-sensitive applications such as IEF or MS. For 1D-SDS-PAGE, you can load flow-through fractions in Buffer A directly.
- **Concentration of flow-through fraction for further analysis**
For further downstream proteomic analysis (e.g. 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. Alternatively, the Agilent mRP-C18 column (part number 5188-5231) may be used for automated desalting and concentration.
- **Lyophilization of flow-through fraction**
If lyophilization of the flow-through fraction (containing the low abundance proteins) is required after recovery from the cartridge, first do buffer exchange to a volatile buffer (such as ammonium bicarbonate). This is recommended due to the high salt concentration of the Buffer A solvent in the flow-through fraction.
- **Bound fraction analysis**
If you wish to analyze the bound fraction, first do 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.

Troubleshooting

Review the following information for troubleshooting your experiments.

Table 3 Troubleshooting suggestions

Problem	Cause	Solution
No flow	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 the Cartridge rewetting section in “Recommendations”).
Incomplete flow (also see <i>No flow</i> solutions, above)	Centrifugation parameters used do not produce the optimal flow rate	Adjust centrifuge force and time to achieve ≤ 0.2 mL/min flow rate through spin cartridge during sample loading on page 13 through Buffer A washes on page 14 .
Backpressure when loading solutions on cartridge	Particulate matter is blocking frits	Filter samples prior to loading. Modify sample preparation methods to reduce amounts of hydrophobic compounds in sample.
No proteins in bound fraction	Buffers A and B reversed	Re-equilibrate spin cartridge with Buffer A (see step 8 on page 14) and start over, using correct buffer sequence.
Breakthrough of high-abundance proteins in flow-through fractions F1 and F2	Cartridge serum capacity exceeded	Reduce serum load per sample.
	Serum protein levels may be unusually high	Reduce serum load per sample.
	Flow rate through cartridge during sample loading too high	Reduce centrifugation force and/or time during sample loading to not exceed 0.2 mL/min flow rate.



3 Reference

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Related Agilent Products 21

This chapter contains reference information.



Spin Cartridge Specifications

Table 4 Specifications for Part Number 5188-5341

Parameter	Description
Type	Affinity depletion spin cartridge
Size	0.45 mL
Cartridge body material	Polypropylene
Frit material	Polyethylene with 35- μ m pore size
Cartridge capacity	14–16 μ L human serum; consult the cartridge Certificate of Analysis.
Recommended centrifugal force	100 $\times g$
Operating temperature	18–25 $^{\circ}$ C
Cartridge packing material	Affinity ligand-modified resin
Immobilized ligands	Affinity ligands to human albumin, IgG, IgA, transferrin, haptoglobin, antitrypsin
Shipping solution	Buffer A with 0.02% sodium azide
Shipping temperature	2–8 $^{\circ}$ C (35–46 $^{\circ}$ F)
Storage temperature	2–8 $^{\circ}$ C (35–46 $^{\circ}$ F)

Related Agilent Products

Agilent Multiple Affinity Removal System spin cartridges and LC columns are listed in [Table 5](#) below.

Table 5 Agilent Multiple Affinity Removal System spin cartridges and LC columns

Product Group	Proteins Removed	Format	Capacity	Part No.
Human-14	albumin, IgG, IgA, transferrin, haptoglobin, antitrypsin, fibrinogen, alpha2-macroglobulin, alpha1-acid glycoprotein, IgM, apolipoprotein AI, apolipoprotein AII, complement C3, transthyretin	spin cartridge	8–10 µL plasma	5188-6560
		4.6 x 50 mm LC column	up to 20 µL plasma	5188-6557
		4.6 x 100 mm LC column	up to 40 µL plasma	5188-6558
		10 x 100 mm LC column	up to 250 µL plasma	5188-6559
Human-7	albumin, IgG, IgA, transferrin, haptoglobin, antitrypsin, fibrinogen	spin cartridge	12–14 µL plasma	5188-6408
		4.6 x 50 mm LC column	30–35 µL plasma	5188-6409
		4.6 x 100 mm LC column	60–70 µL plasma	5188-6410
		10 x 100 mm LC column	250–300 µL plasma	5188-6411
Human-6HC	albumin, IgG, IgA, transferrin, haptoglobin, antitrypsin	spin cartridge	14–16 µL serum	5188-5341
		4.6 x 50 mm LC column	30–40 µL serum	5188-5332
		4.6 x 100 mm LC column	60–80 µL serum	5188-5333
		10 x 100 mm LC column	up to 340 µL serum	5188-5336
Human-6	albumin, IgG, IgA, transferrin, haptoglobin, antitrypsin	spin cartridge	7–10 µL serum	5188-5230
		4.6 x 50 mm LC column	15–20 µL serum	5185-5984
		4.6 x 100 mm LC column	30–40 µL serum	5185-5985
Human-HSA/IgG	albumin, IgG	spin cartridge	up to 50 µL serum	5188-8825
		4.6 x 50 mm LC column	up to 100 µL serum	5188-8826
Human-HSA	albumin	spin cartridge	up to 75 µL serum	5188-5334
		4.6 x 50 mm LC column	up to 175 µL serum	5188-6562
Mouse-3	albumin, IgG, transferrin	spin cartridge	25–30 µL serum	5188-5289
		4.6 x 50 mm LC column	37–50 µL serum	5188-5217
		4.6 x 100 mm LC column	75–100 µL serum	5188-5218

Additional related products for use with the Agilent Multiple Affinity Removal System are listed in [Table 6](#) below.

Table 6 Additional related products

Part Number	Description	Content
5185-5986	Starter Reagent Kit for Multiple Affinity Removal System LC columns	Buffer A: 2 x 1 L
		Buffer B: 1 L
		Spin filters 0.22 µm: 2 packs of 25
		Protein concentrators: 1 pack of 25
5188-5254	Starter Reagent Kit for Multiple Affinity Removal System 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-Lok adapters: 1 pack of 2
		5-mL plastic Luer-Lok syringes: 1 pack of
		1.5-mL microtubes: 6 packs of 100
Spin cartridge extra caps and plugs, 1 pack of 6 each		
5188-5231	mRP-C18 High Recovery Protein Fractionation and Desalting Column	1 Column
		(see www.agilent.com for product details)

www.agilent.com

In This Book

This document describes how to use the Multiple Affinity Removal Spin Cartridge Human-6HC, 0.45 ml to chromatographically remove six interfering high-abundance proteins from human biological samples.

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