Multi Affinity Removal Column, Human-7

Part Nos. 5188-6409 (4.6 × 50 mm)
5188-6410 (4.6 × 100 mm)
5188-6411 (10 × 100 mm)

For depletion of seven high-abundance proteins from human plasma samples

Instructions

Version A1, December 2018

For Research Use Only. Not for use in diagnostic procedures.

Agilent Technologies

Notices

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In this Guide...

This document describes how to use the Multi Affinity Removal Column, Human-7 to chromatographically remove seven interfering high-abundance proteins from human plasma 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 plasma samples and includes troubleshooting information.

3 Reference

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

What's New in Version A1

- Updates to description of needles included with reagent kits (see Table 8 on page 26)
- Correction to part numbers for spin cartridge Starter Reagent Kit, p/n 5188-5254, and mRP-C18 desalting column p/n 5188-5231 (see Table 8 on page 26)
- Updates to Technical Support contact information (see page 2)

Contents

1
Before You Begin

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 Columns, follow general guidelines for handling biological materials and wear protective eyewear and gloves.

Materials Required

The Agilent Multiple Affinity Removal Columns and accessories (purchased separately) used in this protocol are shown in Table 1.

Table 1 Multiple Affinity Removal Column and Accessories

Part number	Product name	Description
5188-6409	Multi Affinity Removal Column Human-7, 4.6 x 50 mm, 1 each	LC column used to remove albumin, IgG, IgA, transferrin, haptoglobin, antitrypsin, and fibrinogen from human plasma samples
5188-6410	Multi Affinity Removal Column Human-7, 4.6 x 100 mm, 1 each	
5188-6411	Multi Affinity Removal Column Human-7, 10 x 100 mm, 1 each	
5185-5987	Buffer A, 1 L	Ready-to-use, optimized buffer for loading, washing, and equilibrating column
5185-5988	Buffer B, 1 L	Ready-to-use, optimized buffer for elution of bound proteins from column
5185-5990	Spin filters, 0.22 µm, 1 pack of 25	For sample cleanup before loading column
5185-5991	Concentrators, 5 kDa MWCO, 1 pack of 25	For concentrating flow-through fractions
5185-5989	Human serum albumin	Dilute standard for checking column capacity (optional)
5190-7995	MARS Column 2 µm Replacement Frit, 2 each	One set of 2 frit assemblies for replacement of clogged inlet and outlet column frits
5185-5986	Starter Reagent Kit	Buffer A: 2 × 1 L Buffer B: 1 L Spin filters 0.22 µm: 2 packs of 25 Protein concentrators: 1 pack of 25

CAUTION

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

Before attaching the column, purge the LC system and run two method blank injections according to protocol to ensure all lines and sample loops are free of organic solvents.

For LC systems shared with other chemical applications, be sure to first purge the LC system, including the sample loop, with isopropyl alcohol, and then extensively with water (approximately 1 hour). After purging, proceed with protocol.

Storage Conditions

Upon its receipt and when you are not using it, store the column with the end-caps tightly sealed at 2°C to 8°C (35°F to 46°F). **Do not freeze the column.**

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 column is specifically designed to remove seven high-abundance proteins from human plasma samples. This technology enables removal of albumin, IgG, IgA, transferrin, haptoglobin, antitrypsin, and fibrinogen with a single device. The targeted high-abundance proteins are simultaneously removed when crude biological samples are passed through the column. Selective immunodepletion provides an enriched pool of low-abundance proteins for downstream proteomics analysis, as depicted in Figure 1 on page 9.

Specific removal of the seven high-abundance proteins depletes approximately 88–92% of total protein mass from human plasma, 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.

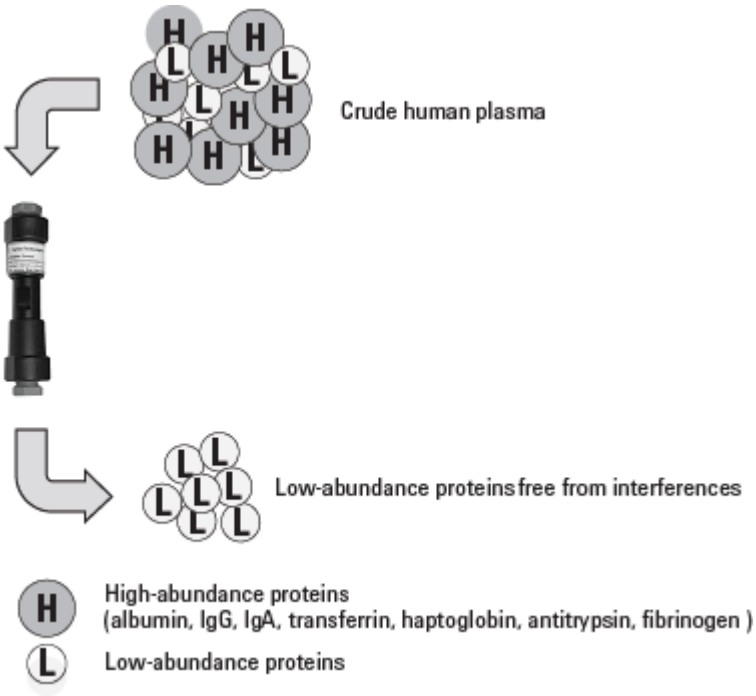

Figure 1 The Multiple Affinity Removal System.

2 Instructions

Protocol for 4.6 x 50 mm column

Column capacity: 30–35 µL human plasma

Step 1. Set up the column (4.6 × 50 mm column)

1 Set up Buffer A and Buffer B as the only mobile phases.

2 Purge lines with Buffer A and Buffer B at a flow rate of 1.0 mL/min for 10 min **without a column**.

3 Set up LC timetable as specified in Table 2.

4 Run two method blanks by injecting 100 µL of Buffer A **without a column**.

5 Ensure that you are using the proper sample loop size in the autosampler, and that the sample loop has been flushed with Buffer A.

6 Attach the column and equilibrate it in Buffer A for 4 min at a flow rate of 1 mL/min at room temperature.

Table 2 LC method for 4.6 x 50 mm column*

Step	Time (min)	%B	Flow Rate (mL/min)	Max. Pressure (bar)
1	0.00	0.00	0.250	120
2	9.00	0.00	0.250	120
3	9.01	100.00	1.000	120
4	12.50	100.00	1.000	120
5	12.60	0.00	1.000	120
6	20.00	0.00	1.000	120

* Solvent A: Buffer A
Solvent B: Buffer B
Detection wavelength: 280 nm

Step 2. Prepare the sample (4.6 × 50 mm column)

Before you begin, consult the Certificate of Analysis for your column to verify the column capacity.

1 Dilute the plasma sample four-fold with Buffer A. For example, if the recommended column loading capacity on the Certificate of Analysis is 35 µL of plasma, dilute 35 µL of plasma with 105 µL Buffer A for a final volume of 140 µL.

   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 serum and CSF with necessary adjustments in sample volume based on albumin concentration.

2 Remove particulates with a 0.22 µm spin filter, spinning for 1 min at 16,000 × *g*.

Step 3. Run the column (4.6 × 50 mm column)

Use the LC timetable (Table 2 on page 11) to complete the following steps.

1 Inject the diluted plasma sample at a flow rate of 0.25 mL/min.

2 Collect the flow-through fraction (like that which appears from 1.5 to 4.5 min in the typical chromatogram shown in Figure 2 on page 13). Store collected fractions at –20 °C if not analyzed immediately.

3 Elute bound proteins from the column with Buffer B (elution buffer) at a flow rate of 1 mL/min for 3.5 min.

4 Regenerate column by equilibrating it with Buffer A for an additional 7.4 min at a flow rate of 1 mL/min.

5 After equilibration with Buffer A, store the column with ends capped at 2°C to 8°C (35°F to 46°F). **Do not freeze the column.**

6 Analyze the flow-through fraction using the guidelines on page 19.

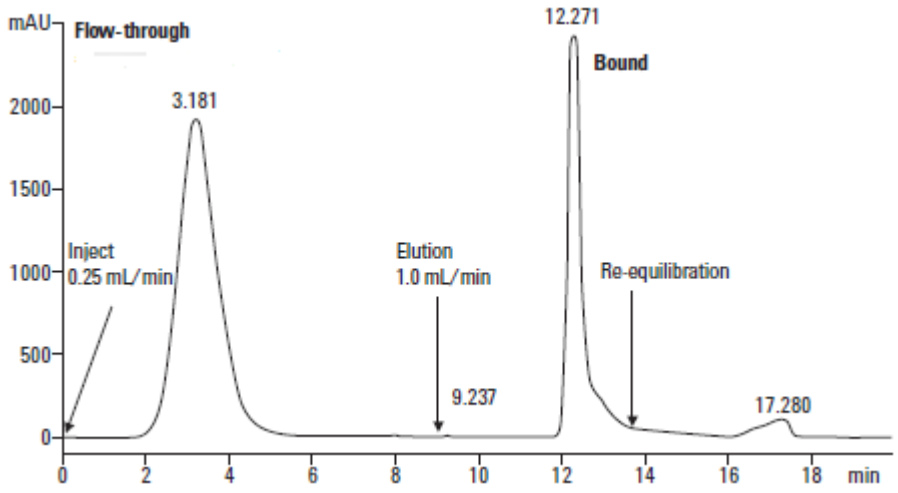

Figure 2 Representative chromatogram for 4.6 x 50 mm column.

Protocol for 4.6 x 100 mm column

Column capacity: 60–70 µL human plasma

Step 1. Set up the column (4.6 × 100 mm column)

1 Set up Buffer A and Buffer B as the only mobile phases.

2 Purge lines with Buffer A and Buffer B at a flow rate of 1.0 mL/min for 10 min **without a column**.

3 Set up LC timetable as specified in Table 3.

4 Run two method blanks by injecting 200 µL of Buffer A **without a column**.

5 Ensure that you are using the proper sample loop size in the autosampler, and that the sample loop has been flushed with Buffer A.

6 Attach the column and equilibrate it in Buffer A for 4 min at a flow rate of 1 mL/min at room temperature.

Table 3 LC method for 4.6 x 100 mm column*

Step	Time (min)	%B	Flow Rate (mL/min)	Max. Pressure (bar)
1	0.00	0.00	0.50	120
2	10.00	0.00	0.50	120
3	10.01	100.00	1.000	120
4	17.00	100.00	1.000	120
5	17.01	0.00	1.000	120
6	28.00	0.00	1.000	120

* Solvent A: Buffer A
Solvent B: Buffer B
Detection wavelength: 280 nm

Step 2. Prepare the sample (4.6 × 100 mm column)

Before you begin, consult the Certificate of Analysis for your column to verify the column capacity.

1 Dilute the plasma sample four-fold with Buffer A. For example, if the recommended column loading capacity on the Certificate of Analysis is 70 µL of plasma, dilute 70 µL of plasma with 210 µL Buffer A for a final volume of 280 µL.

   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 serum and CSF with necessary adjustments in sample volume based on albumin concentration.

2 Remove particulates with a 0.22 µm spin filter, spinning for 1 min at 16,000 × *g*.

Step 3. Run the column (4.6 × 100 mm column)

Use the LC timetable (Table 3 on page 14) to complete the following steps.

1 Inject the diluted plasma sample at a flow rate of 0.5 mL/min.

2 Collect the flow-through fraction (like that which appears from 2.5 to 6 min in the typical chromatogram shown in Figure 3 on page 16). Store collected fractions at –20 °C if not analyzed immediately.

3 Elute bound proteins from the column with Buffer B (elution buffer) at a flow rate of 1 mL/min for 7.0 min.

4 Regenerate column by equilibrating it with Buffer A for an additional 11.0 min at a flow rate of 1 mL/min.

5 After equilibration with Buffer A, store the column with ends capped at 2°C to 8°C (35°F to 46°F). **Do not freeze the column.**

6 Analyze the flow-through fraction using the guidelines on page 19.

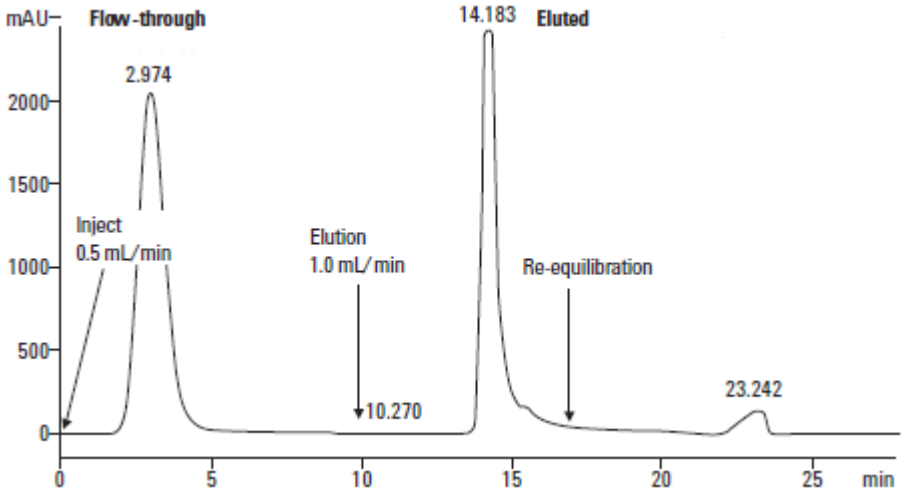

Figure 3 Representative chromatogram for 4.6 x 100 mm column.

Protocol for 10 × 100 mm column

Column capacity: 250–300 µL of human plasma

Step 1. Set up the column (10 × 100 mm column)

1. Set up Buffer A and Buffer B as the only mobile phases.
2. Purge lines with Buffer A and Buffer B at a flow rate of 1.0 mL/min for 10 min **without a column**.
3. Set up LC timetable as specified in Table 4.
4. Run two method blanks by injecting 200 µL of Buffer A **without a column**.
5. Ensure that you are using the proper sample loop size in the autosampler, and that the sample loop has been flushed with Buffer A.
6. Attach the column and equilibrate it in Buffer A for 4 min at a flow rate of 1 mL/min at room temperature.

Table 4 LC method for 10 x 100 mm column*

Step	Time (min)	%B	Flow Rate (mL/min)	Max. Pressure (bar)
1	0.00	0.00	0.500	120
2	20.00	0.00	0.500	120
3	20.01	100.00	3.000	120
4	27.00	100.00	3.000	120
5	27.01	0.00	3.000	120
6	35.00	0.00	3.000	120

* Solvent A: Buffer A
Solvent B: Buffer B
Detection wavelength: 280 nm

Step 2. Prepare the sample (10 × 100 mm column)

Before you begin, consult the Certificate of Analysis for your column to verify the column capacity.

1 Dilute the plasma sample four-fold with Buffer A. For example, if the recommended column loading capacity on the Certificate of Analysis is 300 µL of plasma, dilute 300 µL of plasma with 900 µL Buffer A for a final volume of 1200 µL.

   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 serum and CSF with necessary adjustments in sample volume based on albumin concentration.

2 Remove particulates with a 0.22 µm spin filter, spinning for 1 min at 16,000 × *g*.

Step 3. Run the column (10 × 100 mm column)

Use the LC timetable (Table 4 on page 17) to complete the following steps.

1 Inject the diluted plasma sample at a flow rate of 0.5 mL/min.

2 Collect the flow-through fraction, appearing from 9 to 14 min using the LC method in Table 4 on page 17. Store collected fractions at –20 °C if not analyzed immediately.

3 Elute bound proteins from the column with Buffer B (elution buffer) at a flow rate of 3 mL/min for 7.0 min.

4 Regenerate column by equilibrating it with Buffer A for an additional 8.0 min at a flow rate of 3 mL/min.

5 After equilibration with Buffer A, store the column with ends capped at 2°C to 8°C (35°F to 46°F). **Do not freeze the column.**

6 Analyze the flow-through fraction using the guidelines on page 19.

Guidelines for flow-through fraction analysis

Analyze the flow-through fraction, 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 plasma or other biological samples directly onto the column. Follow instructions for plasma dilution with Buffer A in the protocol for your specific column size.
- **Preventing protein degradation**
  Addition of protease inhibitors to Buffer A for sample dilution helps prevent protein degradation.
- **Sample cleanup**
  Human plasma may contain particulate materials that can be removed by a quick spin using a 0.22-µm spin filter.
- **Variation in column capacity for different samples**
  Concentrations of the proteins targeted for depletion can vary among individual plasma samples and in different types of biological samples. Thus column capacity for samples may differ and you may need to adjust the loading volume for a particular plasma sample.

  For any samples that require adjustment of the plasma load volume, adhere to the instruction to dilute the samples four-fold with Buffer A; do not vary the proportion of plasma and Buffer A in the diluted sample.
- **Column performance**
  Agilent Multiple Affinity Removal Columns should perform reproducibly for greater than 200 runs when handled using the recommended procedures. Buffers A and B are optimized to support column performance and longevity. We cannot guarantee column performance if other buffers are used.

  Do not expose columns to organic solvents (like alcohols, acetonitrile, etc.), strong oxidizers, acids, reducing agents, or other protein-denaturing agents.
- **Column storage**
  To minimize loss in capacity, equilibrate the column with Buffer A. Cap the ends and store at 2°C to 8°C (35°F to 46°F). **Do not freeze the column.**
- **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.

- **Fractionation, desalting, or concentration of flow-through fraction**
  Agilent mRP-C18 column (part number 5188-5231) is recommended for fractionation, desalting, or concentration of flow-through fractions with extremely high protein recoveries. Alternatively, spin concentrators with 5 kDa MWCO (part number 5185-5991) can be used to concentrate proteins before analysis.
- **Lyophilization of flow-through fraction**
  If lyophilization of the flow-through fraction (containing the low abundance proteins) is required after recovery from the column, 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 5 Troubleshooting suggestions

Problem	Cause	Solution
High backpressure **Distorted peak shape** **Diminished column lifetime**	Clogged inlet frits	Remove particulates from samples with a spin filter before loading and replace plugged frits (part number 5190-7995) on both ends of the column.
No bound fraction peak	Salt concentration in diluted sample is not appropriate for affinity binding to column	Sample must be diluted 1:4 in Buffer A to achieve the correct conditions for affinity binding. Follow the recommended sample preparation instructions.
	Insufficient time for exposure of column to Buffer B (elution buffer)	Check LC timetable to ensure enough exposure time to Buffer B for complete removal of bound proteins.
Breakthrough of high-abundance proteins in flow-through fraction	Column plasma capacity exceeded	Reduce plasma load per sample.
	Plasma protein levels may be unusually high	Reduce plasma load per sample.
Abnormal peak height*	Column may not have been regenerated well enough from previous runs, resulting in lost capacity	Elute bound proteins with Buffer B for an additional 3 min and re-equilibrate the column with Buffer A.
	Biological growth in the Buffer A reservoir	Replace with fresh Buffer A.

* Approximately 90% of plasma proteins will be removed as the bound fraction. The peak height of the bound fraction is expected to be greater than that of the flow-through fraction. If the order is reversed, consider the possible causes in the table above.

3 Reference

This chapter contains reference information.

Column Specifications

Table 6 Column specifications

Parameter	Description		
Type	Affinity depletion column		
Part number	5188-6409	5188-6410	5188-6411
Size	4.6 mm × 50 mm (0.83 mL)	4.6 mm × 100 mm (1.66 mL)	10 mm × 100 mm (7.85 mL)
Column capacity*	30–35 µL human plasma	60–70 µL human plasma	250–300 µL human plasma
Column body material	PEEK (polyetheretherketone)		
End-fitting material	PEEK with 2-µm frits		
Maximum pressure	120 bar		
Operating temperature	18–25 °C		
Column packing material	Affinity ligand-modified resin		
Immobilized ligands	Affinity ligands to human albumin, IgG, IgA, transferrin, haptoglobin, antitrypsin, and fibrinogen		
Flow rate range	0.25–3.0 mL/min		
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)		

* Consult the column Certificate of Analysis to verify capacity for plasma samples.

Related Agilent Products

Agilent Multiple Affinity Removal System spin cartridges and LC columns are listed in Table 7 below.

Table 7 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 8 below.

Table 8 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
		Needles, PTFE, Luer-Lok, 1 pack of 10
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 Multi Affinity Removal Column, Human-7 to chromatographically remove interfering high-abundance proteins from human biological samples.

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Agilent Technologies