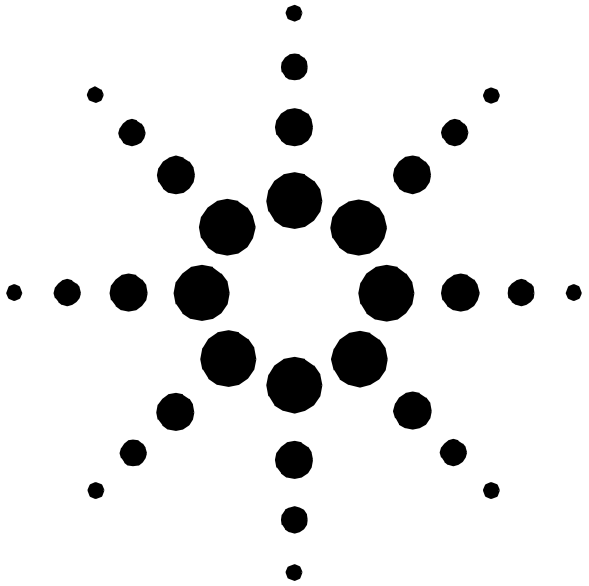

Agilent Human 14 Multiple Affinity Removal System Columns for the Fractionation of High-Abundant Proteins from Human Proteomic Samples

Instructions

Agilent Technologies

General Information

Introduction

The Agilent Human 14 Multiple Affinity Removal System consists of a Multiple Affinity Removal System Column based on affinity interactions and optimized buffers for sample loading, washing, eluting, and regenerating. It is specifically designed to fractionate 14 high-abundant proteins from human biological fluids such as plasma, serum, and cerebral spinal fluid (CSF). This technology enables removal of albumin, IgG, antitrypsin, IgA, transferrin, haptoglobin, fibrinogen, alpha2-macroglobulin, alpha1-acid glycoprotein, IgM, apolipoprotein AI, apolipoprotein AII, complement C3, and transthyretin in a single step. The targeted high-abundant proteins are simultaneously removed when crude biological samples are passed through the column. Selective immunodepletion provides an enriched pool of low-abundant proteins for downstream proteomics analysis.

Specific removal of 14 high-abundant proteins depletes approximately 94% of total protein mass from human serum. The low-abundant proteins in the flow-through fractions can be studied. 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.

Human 14 Multiple Affinity Removal System

The Agilent Human 14 Multiple Affinity Removal System is specially designed to fractionate the high-abundant proteins from the low-abundant proteins, allowing improved analysis of both fractions. (see Figure 1).

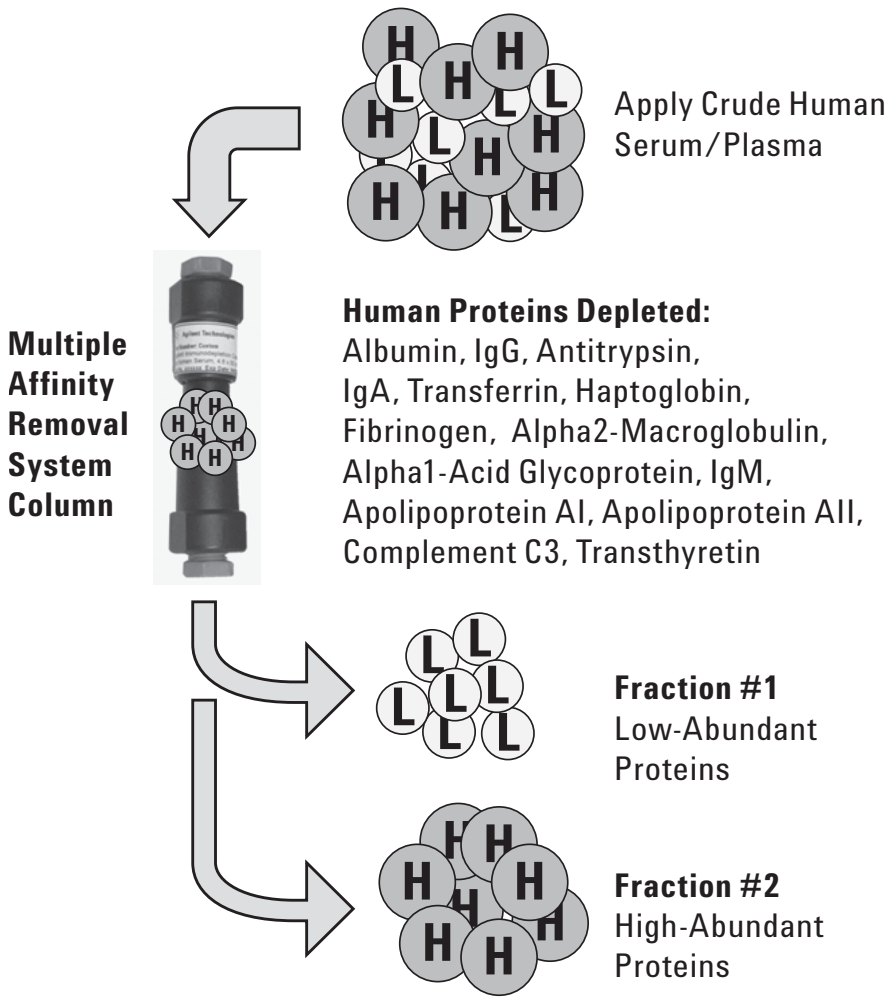

Figure 1 The Multiple Affinity Removal System

Product Description

Table 1 Human 14 Multiple Affinity Removal System Column and Starter Reagent Kit

Product no.	Product name	Product description
5188-6557 5188-6558 5188-6559	4.6 × 50 mm, affinity column 4.6 × 100 mm, affinity column 10 × 100 mm, affinity column	Remove human albumin, IgG, antitrypsin, IgA, transferrin, haptoglobin and fibrinogen, alpha2-macroglobulin, alpha1-acid glycoprotein, IgM, apolipoprotein AI, apolipoprotein AII, complement C3, transthyretin
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

Product no.	Product name	Product description
5185-5995	Replacement end fitting with frit for 4.6 mm ID column (1 each)	Replaced when column inlet frit is clogged.
5021-8814	Replacement end fitting with frit for 10 mm ID column (1 each)	Replace when column inlet frit is clogged.
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	

* Under normal use conditions, the kit should last for approximately 200 injections for 4.6 × 50 mm columns, and 100 injections for 4.6 × 100 mm columns.

CAUTION

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

NOTE

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

Full Protocol for 4.6 x 50 mm Column

(Column capacity: 20 µL human serum/plasma*)

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.
3. Set up LC timetable (see Table 1 for details) and run two method blanks by injecting 100 µL of Buffer A **without a column.**

NOTE Ensure proper sample loop size in autosamplers.

* Consult column certificate of analysis to verify your column capacity.

It is the user's responsibility to adjust loading volume to compensate for the fact that the concentrations of some high-abundant proteins can vary widely depending on the sample origin (source). Several plasma proteins such as alpha1-antitrypsin, haptoglobin, fibrinogen, IgG, and alpha1-acid glycoprotein rise several folds in response to stress, infection, inflammation, or tissue necrosis and are known as acute phase reactants (Henry, J.B. 1996 - Clinical Diagnosis and Management by Laboratory Methods).

Capacity testing was done with pooled normal plasma from Rockland Immunochemicals (D519-04).

Table 2 LC method for 4.6 x 50 mm column

Solvent A: Buffer A	Pressure limits: 60 bar
Solvent B: Buffer B	

LC Timetable

	Time	%B	Flow rate	Max. pressure
1	0.00	0.00	0.125	60
2	9.50	0.00	0.125	60
3	9.51	0.00	1.000	60
4	11.50	0.00	1.000	60
5	11.51	100.00	1.000	60
6	16.00	100.00	1.000	60
7	16.01	0.00	1.000	60
8	25.00	0.00	1.000	60

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

5 Dilute human serum/plasma* 4 times (for example: 20 µL human plasma with 60 µL of Buffer A†).

6 Remove particulates with a 0.22 µm spin filter; 1 min at 16,000 g.

7 Inject 80 µL of the diluted serum/plasma at a flow rate of 0.125 mL/min (injected volume should be adjusted to your column capacity).

8 Collect flow-through fraction (like that which appears between 5.0–7.5 min in Figure 2) and store collected fractions at -20 ° C if not analyzed immediately.

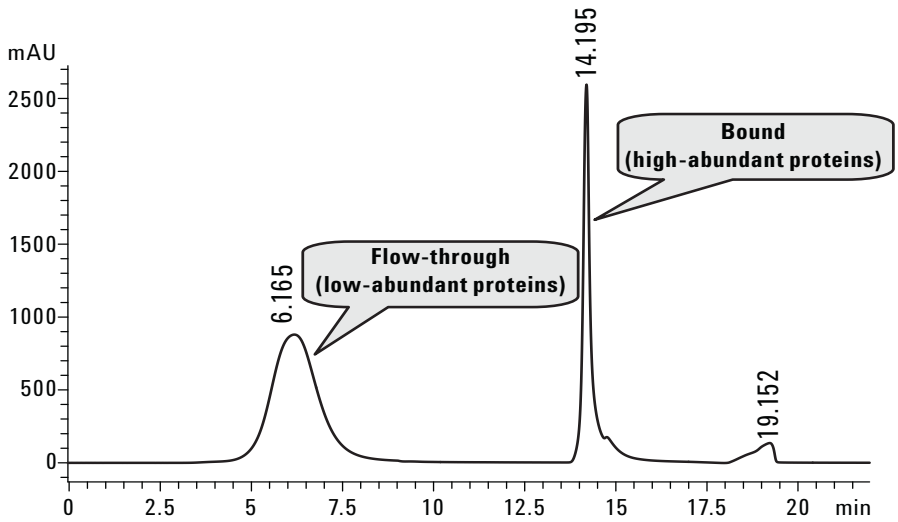

Figure 2 Representative chromatogram of 4.6 x 50 mm column.

* Protocol may be applied to other human biological fluids like CSF.

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

9 Elute bound proteins from the column with Buffer B (elution buffer) at a flow rate of 1 mL/min for 5.5 min.

10 Regenerate column by equilibrating it with Buffer A for an additional 9.0 min at a flow rate of 1 mL/min.

11 Store column after equilibrating with Buffer A at 2–8 °C (35–46 °F) in a refrigerator. DO NOT FREEZE THE COLUMN.

12 **Analyze.** Analyze flow-through 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).

Full Protocol for 4.6 x 100 mm Column

(Column capacity: 40 µL human serum/plasma*)

1. Set up Buffer A and Buffer B as the only mobile phases.
2. Purge LC system 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 (see Table 3 for details) and run two method blanks by injecting 200 µL of Buffer A **without a column**.

NOTE Ensure proper sample loop size in autosamplers.

* Consult column certificate of analysis to verify your column capacity.

It is the user's responsibility to adjust loading volume to compensate for the fact that the concentrations of some high-abundant proteins can vary widely depending on the sample origin (source). Several plasma proteins such as alpha1-antitrypsin, haptoglobin, fibrinogen, IgG, and alpha1-acid glycoprotein rise several folds in response to stress, infection, inflammation, or tissue necrosis and are known as acute phase reactants (Henry, J.B. 1996 - Clinical Diagnosis and Management by Laboratory Methods).

Capacity testing was done with pooled normal plasma from Rockland Immunochemicals (D519-04).

Table 3 LC method for 4.6 x 100 mm column

Solvent A: Buffer A	Pressure limits: 60 bar
Solvent B: Buffer B	

LC Timetable

	Time	%B	Flow rate	Max. pressure
1	0.00	0.00	0.125	60
2	18.00	0.00	0.125	60
3	18.01	0.00	1.000	60
4	20.00	0.00	1.000	60
5	20.01	100.00	1.000	60
6	27.00	100.00	1.000	60
7	27.01	0.00	1.000	60
8	38.00	0.00	1.000	60

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

5 Dilute human serum/plasma* 4 times (for example: 40 µL human plasma with 120 µL of Buffer A†).

6 Remove particulates with a 0.22 µm spin filter; 1 min at 16,000 g.

7 Inject 160 µL of the diluted serum/plasma at a flow rate of 0.125 mL/min (injected volume should be adjusted to your column capacity).

8 Collect flow-through fraction (like that which appears between 11–15 min in Figure 3) and store collected fractions at -20 °C if not analyzed immediately.

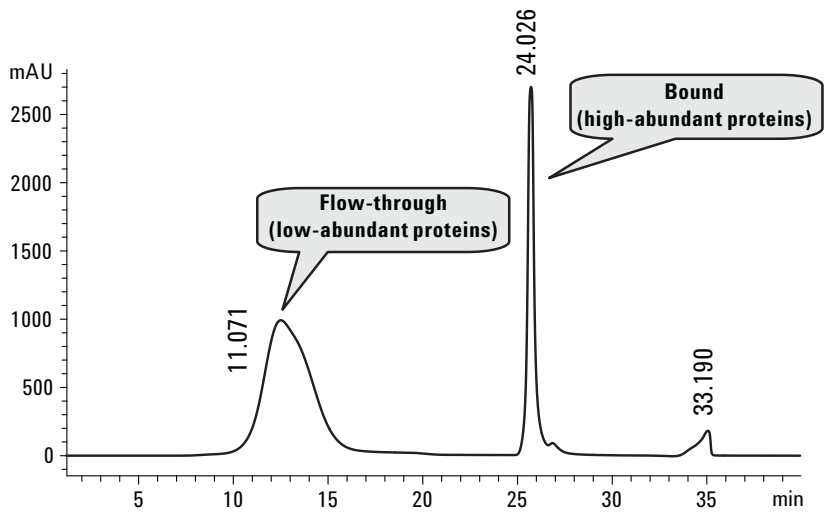

Figure 3 Representative chromatogram of 4.6 x 100 mm column.

* Protocol may be applied to other human biological fluids like CSF.

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

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

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

11 Store column after equilibrating with Buffer A at 2–8 °C (35–46 °F) in a refrigerator. DO NOT FREEZE THE COLUMN.

12 **Analyze**. Analyze flow-through 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).

Full Protocol for 10 x 100 mm Column

(Column capacity: 250 µL human serum/plasma*)

1. Set up Buffer A and Buffer B as the only mobile phases.
2. Purge LC system 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 (see Table 2 for details) and run two method blanks by injecting 200 µL of Buffer A **without a column**.

NOTE

Ensure proper sample loop size in autosamplers.

* Consult column certificate of analysis to verify your column capacity.

It is the user's responsibility to adjust loading volume to compensate for the fact that the concentrations of some high-abundant proteins can vary widely depending on the sample origin (source). Several plasma proteins such as alpha1-antitrypsin, haptoglobin, fibrinogen, IgG, and alpha1-acid glycoprotein rise several folds in response to stress, infection, inflammation, or tissue necrosis and are known as acute phase reactants (Henry, J.B. 1996 - Clinical Diagnosis and Management by Laboratory Methods).

Capacity testing was done with pooled normal plasma from Rockland Immunochemicals (D519-04).

Table 4 LC Method for 10 x 100 mm Column

Solvent A: Buffer A	Pressure limits: 160 bar
Solvent B: Buffer B	

LC Timetable

	Time	%B	Flow rate	Max. pressure
1	0.00	0.00	0.50	160
2	20.00	0.00	0.50	160
3	20.01	0.00	1.000	160
4	22.50	0.00	1.000	160
5	22.51	100.00	3.000	160
6	30.00	100.00	3.000	160
7	30.01	0.00	3.000	160
8	40.00	0.00	3.000	160

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

5 Dilute human serum/plasma* 4 times (for example: 200 µL human plasma with 600 µL of Buffer A†).

6 Remove particulates with a 0.22 µm spin filter; 1 min at 16,000 g.

7 Inject 800 µL of the diluted plasma at a flow rate of 0.5 mL/min (injected volume should be adjusted to your column capacity).

8 Collect flow-through fraction (like that which appears between 10.5–14.5 min in Figure 4) and store collected fractions at -20 °C if not analyzed immediately.

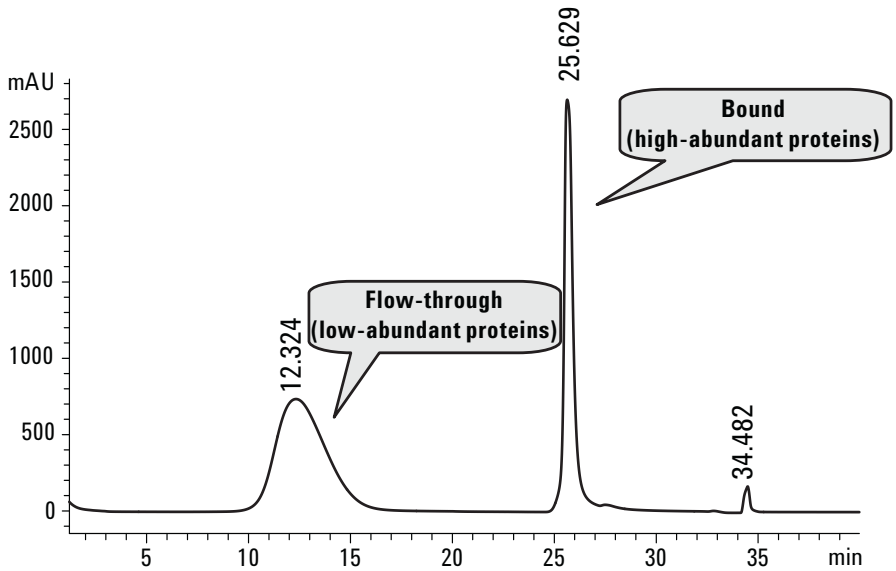

Figure 4 Representative chromatogram of 4.6 x 100 mm column.

* Protocol may be applied to other human biological fluids like CSF.

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

9 Elute bound proteins from the column with Buffer B (elution buffer) at a flow rate of 3 mL/min for 7.5 min.

10 Regenerate column by equilibrating it with Buffer A for 10.0 min at a flow rate of 3 mL/min.

11 Store column after equilibrating with Buffer A at 2–8 °C (35–46 °F) in a refrigerator. DO NOT FREEZE THE COLUMN.

12 **Analyze**. Analyze flow-through 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).

Troubleshooting

It is the user's responsibility to adjust loading volume to compensate for the fact that the concentrations of some high-abundant proteins can vary widely depending on the sample origin (source). Several plasma proteins such as alpha1-antitrypsin, haptoglobin, fibrinogen, IgG, and alpha1-acid glycoprotein rise several folds in response to stress, infection, inflammation, or tissue necrosis and are known as acute phase reactants (Henry, J.B. 1996 - Clinical Diagnosis and Management by Laboratory Methods).

Review the following information for troubleshooting your experiments.

High backpressure

A clogged inlet frit may result in high backpressure, distorted peak shape, and diminished column lifetime. To prevent these problems, remove particulates from samples with a spin filter before loading. If clogging occurs, replace plugged inlet frit (Part numbers: 5185-5995 for 4.6 mm ID columns and 5021-8814 for 10 mm ID columns).

No bound fraction peak

Bound proteins can only be removed from the column by eluting with Buffer B. Check LC timetable to ensure enough column time exposure to Buffer B, for complete removal of bound proteins.

Abnormal peak height

Approximately 94% of serum/plasma proteins will be removed as the bound fraction. The peak height of the bound fraction is expected to be much greater than that of the flow-through fraction. If this order is reversed, two possibilities may be checked:

- **a** Column may not have been regenerated well enough from previous runs, resulting in lost capacity. To correct this, elute bound proteins with Buffer B for 3 additional minutes and re-equilibrate the column with Buffer A.
- **b** Check for signs of biological growth in the Buffer A reservoir. Replace with fresh Buffer A for optimal column performance.

Breakthrough of high-abundant proteins in flow-through fraction

The majority (95–99%) of targeted proteins should be depleted from the sample. If the flow-through fraction contains unusually high levels of the 14 depleted proteins, two possibilites may be checked:

- **a** The column capacity may have been exceeded. Reduce the sample load per injection.
- **b** Serum/plasma levels of the targeted proteins may be unusually high.

Column Specifications

Table 5 Column Descriptions

Parameter	Description
Part number 5188-6557	4.6 mm × 50 mm (0.83 mL)
Part number 5188-6558	4.6 mm × 100 mm (1.66 mL)
Column body material	Polyetheretherketone (PEEK)
Part Number 5188-6559	10 mm × 100 mm (7.85 mL)
Column body material	PEEK encapsulated in stainless steel
End-fitting materials	PEEK with 2 µm frits
Column capacity*	0.83 mL column: 20 µL human plasma 1.66 mL column: 40 µL human plasma 7.85 mL column: 250 µL human plasma
Max. pressure	60 or 160 bar

Table 5 Column Descriptions

Parameter	Description
Operating temperature	18–25 °C
Column packing material	Affinity resin
Immobilized ligands	Affinity ligands to human albumin, IgG, antitrypsin, IgA, transferrin, haptoglobin and fibrinogen, alpha2-macroglobulin, alpha1-acid glycoprotein, IgM, apolipoprotein AI, apolipoprotein AII, complement C3, transthyretin
Flow rate range	0.125–3.0 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)

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

Safety Issues

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

Recommendations

- **Sample dilution**
  It is not recommended to load crude serum/plasma directly onto the column. Follow instructions for serum/plasma dilution (four times dilution with Buffer A). Addition of protease inhibitors in Buffer A for sample dilution helps prevent protein degradation.
- **Sample cleanup**
  Human serum/plasma may contain particulate materials that can be removed by a quick spin using a 0.22-µm spin filter.
- **Column performance**
  Always use Buffers A and B as the mobile phases according to the LC protocols. Do not expose the columns to solvents other than Buffers A and B.
- **Column storage**
  After equilibrating Agilent Multiple Affinity Removal System Columns with Buffer A, always store them in a refrigerator at 2–8 °C (35–46 °F) when not in use to minimize loss in column 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.
- **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).

CAUTION Do not expose columns 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: Upon its receipt and when you are not using it, store the column with the end-caps tightly sealed in a refrigerator at 2–8 °C (35–46 °F). DO NOT FREEZE THE COLUMN.

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

Related Agilent Products

Other related Agilent products include the following:

5188-6560 **Agilent Multiple Affinity Removal System Spin Cartridge Hu-14,** spin cartridge that depletes 14 high-abundant proteins (albumin, IgG, IgA, transferrin, haptoglobin, antitrypsin, fibrinogen, alpha2-macroglobulin, alpha1-acid glycoprotein, IgM, apolipoprotein AI, apolipoprotein AII, complement C3, and transthyretin) from human serum/plasma samples, 8–10 µL serum/plasma capacity per use

5188-6409 **Agilent High Capacity Multiple Affinity Removal System Column Hu-7HC, 4.6 X 50 mm**, LC column that depletes seven high-abundant proteins (albumin, IgG, IgA, transferrin, haptoglobin, antitrypsin, and fibrinogen) from human serum or plasma samples, 30–35 µL serum/plasma capacity per injection

5188-6410 **Agilent High Capacity Multiple Affinity Removal System Column Hu-7HC, 4.6 X 100 mm**, LC column that depletes seven high-abundant proteins (albumin, IgG, IgA, transferrin, haptoglobin, antitrypsin, and fibrinogen) from human serum or plasma samples, 60–70 µL serum/plasma capacity per injection

5188-6411 **Agilent High Capacity Multiple Affinity Removal System Column Hu-7HC, 10 X 100 mm**, LC column that depletes seven high-abundant proteins (albumin, IgG, IgA, transferrin, haptoglobin, antitrypsin, and fibrinogen) from human serum or plasma samples, 250–300 µL serum/plasma capacity per injection

5188-6408 **Agilent High Capacity Multiple Affinity Removal System Spin Cartridge Hu-7HC**, spin cartridge that depletes seven high-abundant proteins (albumin, IgG, IgA, transferrin, haptoglobin, antitrypsin, and fibrinogen) from human serum or plasma samples, 12–14 µL serum/plasma capacity per use

5188-5332 **Agilent High Capacity Multiple Affinity Removal System Column Hu-6HC, 4.6 X 50 mm**, LC column that depletes six high-abundant proteins (albumin, IgG, IgA, transferrin, haptoglobin, and antitrypsin) from human serum/plasma samples, 30–40 µL serum capacity per injection

5188-5333 **Agilent High Capacity Multiple Affinity Removal System Column Hu-6HC, 4.6 X 100 mm**, LC column that depletes six high-abundant proteins (albumin, IgG, IgA, transferrin, haptoglobin, and antitrypsin) from human serum/plasma samples, 60–80 µL serum capacity per injection

5188-5336 **Agilent High Capacity Multiple Affinity Removal System Column Hu-6HC, 10 X 100 mm**, LC column that depletes six high-abundant proteins (albumin, IgG, IgA, transferrin, haptoglobin, and antitrypsin) from human serum/plasma samples, 300–325 µL serum capacity per injection

5188-5341 **Agilent High Capacity Multiple Affinity Removal System Spin Cartridge Hu-6HC**, spin cartridge that depletes six high-abundant proteins (albumin, IgG, IgA, transferrin, haptoglobin, and antitrypsin) from human serum/plasma samples, 14–16 µL serum capacity per use

5185-5984 **Multiple Affinity Removal System Column Hu-6, 4.6 × 50 mm**, LC column that depletes six high-abundant proteins (albumin, IgG, IgA, transferrin, haptoglobin, and antitrypsin) from human serum/plasma samples, 15–20 µL serum capacity per injection

5185-5985 **Multiple Affinity Removal System Column Hu-6, 4.6 × 100 mm**, LC column that depletes six high-abundant proteins (albumin, IgG, IgA, transferrin, haptoglobin, and antitrypsin) from human serum/plasma samples, 30–40 µL serum capacity per injection

5188-2714 **Multiple Affinity Removal System Column Hu-6, 10 × 100 mm**, LC column that depletes six high-abundant proteins (albumin, IgG, IgA, transferrin, haptoglobin, and antitrypsin) from human serum/plasma samples, 140–190 µL serum capacity per injection

5188-5230 **Multiple Affinity Removal System Spin Cartridge Hu-6**, spin cartridge that depletes six high-abundant proteins (albumin, IgG, IgA, transferrin, haptoglobin, and antitrypsin) from human serum/plasma samples, 7–10 µL serum capacity per use

5188-5217 **Multiple Affinity Removal System Column Ms-3, 4.6 × 50 mm**, LC column that depletes three high-abundant proteins (albumin, IgG, and transferrin) from mouse serum/plasma samples, 37–50 µL serum capacity per injection

5188-5218 **Multiple Affinity Removal System Column Ms-3, 4.6 × 100 mm,** LC column that depletes three high-abundant proteins (albumin, IgG, and transferrin) from mouse serum/plasma, 75–100 µL serum capacity per injection

5188-5289 **Multiple Affinity Removal System Spin Cartridge Ms-3,** spin cartridge that depletes three high-abundant proteins (albumin, IgG, and transferrin) from mouse serum/plasma samples, 25–30 µL serum capacity per use

5185-5986 **Multiple Affinity Removal System Reagent Kit**, starter reagent kit containing buffers, spin filters, and spin concentrators for use with Multiple Affinity Removal System LC Columns

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

5188-5231 **mRP-C18 High Recovery Protein Fractionation and Desalting Column**, see www.agilent.com/chem/bioreagents for more details

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

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

Printed in the USA
May 2007
5969-1596

Agilent Technologies