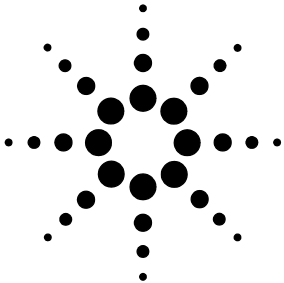

Agilent Multiple Affinity Removal Columns – for Mouse Serum Proteins

Instructions

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Agilent Technologies

General Information

Introduction

The Multiple Affinity Removal System for Mouse Serum Proteins consists of:

- A multiple affinity removal column based on antibody-antigen interactions
- Optimized buffers for sample loading, washing, eluting and regenerating columns
- Spin filters
- Protein concentrators

This technology removes albumin, IgG, and transferrin (high-abundant proteins) from mouse (murine) biological fluids in a single step. When crude biological samples, such as serum and plasma, are passed through the column, immobilized antibodies remove the targeted high-abundant proteins. Selective immunodepletion provides an enriched pool of low-abundant proteins for downstream proteomics analysis. Figure 1 depicts this process.

Specific removal of these high-abundant proteins depletes approximately 80% of total protein mass from mouse serum. This allows the remaining low-abundant proteins to 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 mass spectrometry. You may need to concentrate and buffer-exchange the collected flow-through fractions depending upon the downstream applications.

Overview

Figure 1 Overview of the system.

Product Description

The Multiple Affinity Removal Columns come in two sizes (see Table 1).

Table 1 Column descriptions

Product no.	Product name	Product description
5188-5217	4.6 x 50-mm affinity column	37–50-µL serum capacity* Removes mouse albumin, IgG, and transferrin
5188-5218	4.6 x 100-mm affinity column	75–100-µL serum capacity* Removes mouse albumin, IgG, and transferrin

*Determined with pooled Swiss Webster mouse serum; results may vary with the mouse strain used.

There is also a separate Starter kit, which contains the necessary buffers, filters, and concentrators that are required for processing samples (see Table 2).

Table 2 Starter kit description

Product no.	Product description
5185-5986	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

You can also order the contents of the Starter kit individually (see Table 3).

Table 3 Starter kit contents

Product no.	Product name	Product description
5185-5987	Buffer A 1 L	Ready-to-use, optimized buffer for loading, washing and equilibration
5185-5988	Buffer B 1 L	Ready-to-use, optimized buffer for elution of bound proteins
5185-5990	Spin filters 0.22 µm, 1 pack of 25	For sample cleanup before loading
5185-5991	Concentrators 1 pack of 25 4-mL, 5 kDa MWCO	For concentrating flow-through fractions

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

Protocol for 4.6 x 50-mm Column

(Column capacity: 37–50 µL mouse serum*)

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 the LC timetable (see Table 4 for details) and run two method blanks by injecting 100 µL of Buffer A **without a column**.
4. Ensure proper sample loop size in autosamplers.
5. Attach the column and equilibrate in Buffer A for 4 min at a flow rate of 1 mL/min at room temperature.
6. Dilute the mouse serum five times (for example: 40-µL mouse serum with 160 µL of Buffer A**).
7. Remove particulates with a 0.22-µm spin filter; 1 min at 16,000 x g.
8. Inject 200–225 µL of the diluted serum (see step 6, above) at a flow rate of 0.25 mL/min (adjust the injected volume to your column capacity).
9. Collect the flow-through fraction (like that which appears between 3–6 min in Figure 2). Store collected fractions at –20 °C if not analyzed immediately.
10. Elute bound proteins from the column with Buffer B (elution buffer) at a flow rate of 1 mL/min for 3.5 min.
11. Regenerate the column by equilibrating it with Buffer A for 7.4 min at a flow rate of 1 mL/min.

12 Equilibrate with Buffer A and then store the column with the ends capped in a refrigerator at 2–8 °C (35–46 °F). DO NOT FREEZE THE COLUMN.

13 **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).

* Consult the column certificate of analysis to verify your column capacity. The capacity range was determined with pooled Swiss Webster mouse serum and tests have shown 43-µL capacity at 98%+ removal efficiency using the 50-mm column. Mouse serum protein concentrations can vary for other strains and your injected sample size should be adjusted accordingly.

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

Table 4 LC method for 4.6 x 50-mm column

LC Timetable

Event	Time (min)	% Buffer 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
Pressure limits: 120 bar

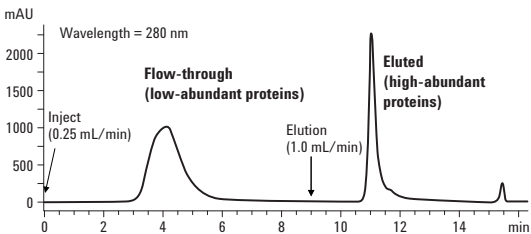

Figure 2 Chromatogram of 4.6 x 50-mm column

Protocol for 4.6 x 100-mm Column

(Column capacity: 75–100 µL mouse serum*)

1. Set up Buffer A and Buffer B as the only mobile phases.
2. Purge the 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 the LC timetable (see Table 5 for details) and run two method blanks by injecting 200 µL of Buffer A **without a column**.
4. Ensure proper sample loop size in autosamplers.
5. Attach the column and equilibrate in Buffer A for 4 min at a flow rate of 1 mL/min at room temperature.
6. Dilute the mouse serum five times (for example: 90-µL mouse serum with 360-µL of Buffer A**).
7. Remove particulates with a 0.22-µm spin filter; 1 min at 16,000 x g.
8. Inject 450–500 µL of the diluted serum (see step 6, above) at a flow rate of 0.5 mL/min (adjust the injected volume to your column capacity).
9. Collect the flow-through fraction (like that which appears between 3–7.0 min in Figure 3) and store collected fractions at –20 °C if not analyzed immediately.
10. Elute bound proteins from the column with Buffer B (elution buffer) at a flow rate of 1 mL/min for 7.0 min.
11. Regenerate the column by equilibrating it with Buffer A for 11.0 min at a flow rate of 1 mL/min.

12 Equilibrate with Buffer A, and then store the column, with the ends capped, in a refrigerator at 2–8 °C (35–46 °F). DO NOT FREEZE THE COLUMN.

13 **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).

* Consult the column certificate of analysis to verify your column capacity. The capacity range was determined with pooled Swiss Webster mouse serum and tests have shown 100-µL capacity at 98%+ removal efficiency using the 100-mm column. Mouse serum protein concentrations can vary for other strains and your injected sample size should be adjusted accordingly.

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

Table 5 LC method for 4.6 x 100-mm column

LC Timetable

Event	Time (min)	% Buffer B	Flow rate (mL/min)	Max. pressure (bar)
1	0.00	0.00	0.500	120
2	10.00	0.00	0.500	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
Pressure limits: 120 bar

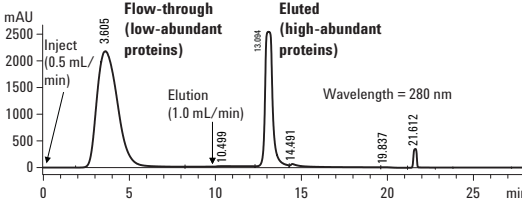

Figure 3 Chromatogram of 4.6 x 100-mm column

Troubleshooting

Review the following information for troubleshooting your experiments.

High backpressure

Clogged inlet frits 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 and then replace the plugged inlet frit (Product # 5185-5995).

No bound fraction peak

Bound proteins can only be removed from the column by eluting with Buffer B. Check the LC timetable to ensure enough elution time with Buffer B, which completely removes bound proteins.

Abnormal peak height

Approximately 80% of serum proteins will be removed in the bound fraction. The peak height of the bound fraction is expected to be greater than that of the flow-through fraction. If this order is reversed, two possibilities may be checked:

- 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.
- Check for signs of biological growth in the Buffer A reservoir. Replace with fresh Buffer A for optimized column performance.

Column Specifications

Product #5188-5217	4.6 mm x 50 mm (0.83 mL)
Product #5188-5218	4.6 mm x 100 mm (1.66 mL)
Column body material	PEEK (polyetheretherketone)
End-fitting material	PEEK with 2-µm frits
Max. pressure	120 bar
Operating temperature	18–25°C
Column packing material	Antibody-modified resin
Immobilized ligands	Affinity-purified polyclonal antibodies to mouse albumin, IgG, and transferrin
Flow rate range	0.25–1.0 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)

Safety Issues

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

Recommendations

Sample dilution

Do not load crude serum directly onto the column. Follow instructions for serum dilution (five times dilution with Buffer A). 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.

Column performance

Multiple Affinity Removal Columns should perform reproducibly for at least 200 runs under proper conditions. 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 a loss in capacity, equilibrate the column with Buffer A. Then, cap the ends and store in a refrigerator at 2–8 °C (35–46 °F).

Lyophylization of flow-through fractions

Due to a high salt concentration in Buffer A, buffer exchanging to a volatile buffer before lyophylization is recommended. An example of such a volatile buffer is one containing ammonium bicarbonate.

Analysis of flow-through fractions

Buffer exchanging 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. Protein analysis of bound fractions requires a buffer exchange.

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

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