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Product Information

Seppro® Mouse SuperMix LC5 Column

Catalog Number **SEP100**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description
Immunoaffinity partitioning of highly abundant proteins (HAP) has proven to be one of the most effective approaches for overcoming the wide dynamic range of protein concentration in biological samples. However, after the removal of the HAP using the Seppro® Mouse LC10 column, the remaining moderately abundant proteins (MAP) become obstacles to access the low abundance proteins (LAP), in which the majority of biologically interesting and clinically important biomarkers reside. After the use of Mouse LC10 column, the SuperMix further partitions complex plasma/serum samples.

Chickens are immunized with mouse plasma proteins with 7 HAP removed. The majority of resultant antibodies incorporated into the SuperMix resin are against MAP and/or immunoreactive proteins. The Mouse SuperMix LC5 column is designed for use in conjunction with Mouse LC10 column to enable the detection of low abundance proteins.

The Mouse LC10 column is specifically designed to remove seven highly abundant proteins from mouse biological fluids such as serum or plasma. The following proteins are depleted in a single step:

Albumin	IgG
α_1-Antitrypsin	IgM
Transferrin	Haptoglobin
Fibrinogen	

The immobilized specific IgY antibodies simultaneously remove the targeted 7 HAP when crude biological samples are passed through the column. Specific removal of these seven highly abundant proteins depletes 60–70% of the total protein mass from mouse serum or plasma.

After the removal of 7 HAP, the sample is continuously passed through the Mouse SuperMix LC5 column. The flow-through fraction after both columns contains ~1% of total protein mass. Both the collected flow-through fraction (~1% of total plasma protein) and bound fraction can be used for downstream analysis.

Characteristics of the Mouse LC10 and Mouse SuperMix LC5 Columns

Size:
Mouse LC10 – 12.7 × 79.0 mm
(10 ml bed volume)
Mouse SuperMix LC5 – 12.7 × 39.5 mm
(5 ml bed volume)

Capacity:
Mouse LC10: 4.5 mg of total protein or ~112 µl of mouse plasma based on an average protein concentration of 40 mg protein/ml.

Mouse SuperMix LC5: Flow through volume from Mouse LC10

Total protein mass removal after two columns: ~99%

Maximum Operation Pressure: 350 psi (21 bars)
Antibody-modified resin only withstands 100 psi

Flow rate: 0.5–2.0 ml/minute

Operating temperature: 18–25 °C

Shipping Buffer: 1× Dilution Buffer with 0.02% sodium azide

Column body materials: Polycarbonate column cylinder, Polyethylene frit, Tefzel® caps, Buna-N-rubber O- rings, Delrin® nut fittings, ETFE ferrules, and PTFE PFA tubing.

Usage: Columns may be used 100 times.

Components

Seppro Mouse SuperMix LC5 Column (Catalog Number S5824)	1 each
10× Dilution Buffer Tris-Buffered Saline (TBS) - 100 mM Tris-HCl with 1.5 M NaCl, pH 7.4 (Catalog Number S4199)	2 × 200 ml
10× Stripping Buffer 1 M Glycine, pH 2.5 (Catalog Number S4324)	2 × 200 ml
10× Neutralization Buffer 1 M Tris-HCl, pH 8.0 (Catalog Number S4449)	2 × 80 ml

Equipment Required for Depletion, but Not Provided

Seppro Mouse LC10 Column
(Catalog Number S5699)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Preparation of 1× concentration buffers - Separately dilute the three 10× buffers (Dilution, Stripping, and Neutralization Buffers) 10-fold with water. If precipitation occurs in the 10× buffers, allow the bottle to warm to room temperature and mix until completely dissolved prior to use. **Do not dilute all of the 10× Neutralization Buffer,** save a volume of the 10× neutralization buffer for neutralization of eluted bound proteins if analysis of bound proteins is desired.

Sample Preparation - It is not recommended to load unfiltered serum or plasma directly onto the columns. Mouse serum or plasma samples should be diluted 5-fold with 1× Dilution Buffer. Samples may contain particulate materials, which can be removed with a 0.45 μm spin filter, centrifuge for 1 minute at 9,000 × *g*.

Storage/Stability

Store the column at 2–8 °C. After use, equilibrate the column with 1× Dilution Buffer containing 0.02% sodium azide and store the column at 2–8 °C with the end-caps tightly sealed. **Do Not Freeze** the column.

Procedure

Note: Always use the three 1× buffers as the mobile phases for the LC procedure. Adjust the LC procedure appropriately for the instrumentation being used. Do not expose the column to solvents other than the three 1× buffers. Do not expose the column to organic solvents (like alcohols, acetonitrile, etc.), strong oxidizers, acids, or reducing agents and other protein denaturing agents (urea).

Preparation of the Liquid Chromatography System

1. Set up the three 1× buffers as the only mobile phases.
2. Purge lines with the three 1× buffers and run the 1× Dilution Buffer at 2 ml/minute without a column to check the system backpressure.
   Note: The maximum operation pressure includes the pressure introduced by the column and the system backpressure from the instrument. Usually, the pressure introduced by the column is less than 50 psi. It is important to first check the system backpressure of the instrument before using these columns. If the system backpressure is more than 300 psi, use tubing with a larger I.D. or even change the flow-cell to tubing with a larger I.D. to reduce the system backpressure.
3. Before processing any samples, both the Mouse LC10 and Mouse SuperMix LC5 columns need to be subjected to two buffer runs using standard LC10 and LC5 procedures. Run two buffer blanks by injecting 1,250 μl and 500 μl of 1× Dilution Buffer through the LC10 and LC5 columns, respectively.

Depletion Process with Linear Connection between LC10 and LC5 Columns

1. For LC systems without a control valve, couple the columns with a linear connection between the Mouse LC10 and Mouse SuperMix LC5 columns.
2. After obtaining a flat baseline from the two coupled columns, load the diluted and filtered sample. Run 1× Dilution Buffer only at 0.5 ml/minute for 15 minutes and then 1.5 ml/minute for 10 minutes for a total of 25 minutes to get the flow-through fraction, which has passed both the Mouse LC10 and Mouse SuperMix LC5 columns.

Elution of Bound Proteins from Mouse LC10

1. Connect the Mouse LC10 column alone to the system. Start with a wash of 1× Dilution Buffer, elute the bound proteins with 1× Stripping Buffer, followed by 1× Neutralization Buffer, and regenerate the column with 1× Dilution Buffer according to the LC Timetable (see Table 1) for Mouse LC10 column.
2. Store collected fractions at –70 °C if not analyzed immediately.
   Note: Due to high salt concentration in the 1× Dilution Buffer, buffer exchange of the flow-through fractions to a volatile buffer (for example, ammonium bicarbonate) is recommended prior to lyophilization.
   Note: Neutralize the eluted fractions with 0.1× fraction volume of 10× Neutralizing Buffer if further analysis of bound fractions is desired.

Table 1.
Timetable for the elution of bound proteins from Mouse LC10 column

Cycle	Time (minutes)	1× Dilution Buffer (%)	1× Stripping Buffer (%)	1× Neutralization Buffer (%)	Flow rate (ml/minute)	Maximum pressure (psi)
Injection						
Wash	0	100	0	0	2.0	350
Elution	4.01	0	100	0	2.0	350
Neutralization	25.01	0	0	100	2.0	350
Re-equilibrium	35.01	100	0	0	2.0	350
Stop	45					

Elution of Bound Proteins from Mouse SuperMix LC5

1. Disconnect the Mouse LC10 column and connect the Mouse SuperMix LC5 to the system. Start with a wash of 1× Dilution Buffer, elute the bound proteins with 1× Stripping Buffer, followed by 1× Neutralization Buffer, and regenerate the column with 1× Dilution Buffer according to the LC Timetable (see Table 2) for Mouse SuperMix LC5 column.
2. Store collected fractions at –70 °C if not analyzed immediately.
   Note: Due to high salt concentration in the 1× Dilution Buffer, buffer exchange of the flow-through fractions to a volatile buffer (for example, ammonium bicarbonate) is recommended prior to lyophilization.
   Note: Neutralize the eluted fractions with 0.1× fraction volume of 10× Neutralizing Buffer if further analysis of bound fractions is desired.

Table 2.
Timetable for the elution of bound proteins from Mouse SuperMix LC5 column

Cycle	Time (minutes)	1× Dilution Buffer (%)	1× Stripping Buffer (%)	1× Neutralization Buffer (%)	Flow Rate (ml/minute)	Maximum Pressure (psi)
Injection						
Wash	0	100	0	0	2.0	350
Elution	5.01	0	100	0	2.0	350
Neutralization	20.01	0	0	100	2.0	350
Re-equilibration	27.01	100	0	0	2.0	350
Stop	35.00					

Note: If a control valve is available, depletion, elution of bound proteins from the Mouse LC10 column, and elution of bound proteins from SuperMix LC5 column can be integrated into one method based on parameters described for each method.

Reference

1. Huang, L. et al., Immunoaffinity separation of plasma proteins by IgY microbeads: Meeting the needs of proteomic sample preparation and analysis. Proteomics, **5**, 3314-3328 (2005).

Appendix

Troubleshooting Guide

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.

No bound fraction peak - Bound proteins can only be removed from the column by eluting with 1× Stripping Buffer. Check LC timetable to ensure enough column exposure time to the 1× Stripping Buffer for complete removal of bound proteins.

Abnormal peak height - 95–99% 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:

- Column may not have been regenerated properly after previous use, resulting in lost capacity. To correct this, elute bound proteins with 2 additional column volumes of 1× Stripping Buffer and then neutralize and re-equilibrate the column with 1× Neutralizing Buffer and 1× Dilution Buffer.

- Check for signs of biological growth in the buffer reservoirs. Replace with fresh buffers for optimized column performance.

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