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Product Information

Seppro® Rat LC10 Column

Catalog Number **SEP120**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

The Seppro® Rat Liquid Chromatography 10 (LC10) Column is based on avian antibody (IgY)-antigen interactions. Optimized buffers for sample application, and washing, elution, and regeneration of the column are provided. The column is specifically designed to remove seven highly abundant proteins from rat 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 targeted highly abundant proteins are simultaneously removed by the immobilized specific IgYs when crude biological samples are passed through the column.

Selective immunodepletion provides an enriched pool of low abundance proteins for downstream proteomic analyses. Specific removal of these seven highly abundant proteins depletes >80% of the total protein mass from rat serum or plasma. The low abundance proteins in the flow-through fractions can then be studied. Removal of highly abundant proteins enables improved resolution and dynamic range for one dimensional electrophoresis (1DE), two dimensional (2DE) electrophoresis, and liquid chromatography/mass spectrometry (LC/MS). The collected flow-through fractions may need to be concentrated dependent upon the downstream application.

Characteristics of the Rat LC10 Column

Size: 12.7 × 79.0 mm (10 ml bed volume)

Capacity: 12 mg of total protein or ~266 µl of rat plasma based on an average protein concentration of 45 mg protein/ml.
Note: If the protein concentration of the sample is unknown and the total serum protein levels are potentially elevated, a reduction of the serum load to 186.2 µl is recommended for initial study to avoid potential abundant protein bleed through.

Total protein mass removal: 60-70%

Targeted depletion efficiency: 85% (average)

Maximum 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: Column may be used 100 times.

Components

Seppro Rat LC10 Column (Catalog Number S6074)	1 each
10× Dilution Buffer Tris-Buffered Saline (TBS) - 100 mM Tris-HCl with 1.5 M NaCl, pH 7.4 (Catalog Number S4199)	3 × 200 ml

10× Stripping Buffer 1 M Glycine, pH 2.5 (Catalog Number S4324)	3 × 200 ml
10× Neutralization Buffer 1 M Tris-HCl, pH 8.0 (Catalog Number S4449)	3 × 80 ml
Corning® Spin-X® Centrifuge Tube Filters 0.45 µm, pack of 100 (Catalog Number CLS8163)	1 pack

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 directly onto the column. Rat serum 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).

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 this column. 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. Attach the column to chromatography instrument (see Appendix) and equilibrate it with 1× Dilution Buffer for 20 minutes at a flow rate of 2.0 ml/minute to obtain a flat baseline.
4. Set up a LC timetable (see Table 1) and run two buffer blanks by injecting 1,250 µl of 1× Dilution Buffer.
   Note: Adjust LC timetable based on instrumentation available, if necessary.
5. Inject 750 µl of the diluted and filtered plasma or serum (see Sample Preparation), start with a flow rate of 0.5 ml/minute for 30 minutes, wash the column at a flow rate of 2.0 ml/minute for 5 minutes, collect flow-through fraction, and 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.
6. Elute bound proteins from the column with 1× Stripping Buffer at a flow rate of 2.0 ml/minute for 20 minutes and neutralize the eluted fractions with 0.1× fraction volume of 10× Neutralizing Buffer.
   Note: Do not expose the column to the 1× Stripping Buffer for more than 20 minutes.
7. Neutralize the column with 1× Neutralizing Buffer at a flow rate of 2.0 ml/minute for 10 minutes.
8. Re-equilibrate the column with 1× Dilution Buffer for an additional 10 minutes at a flow rate of 2.0 ml/minute. The re-equilibrated column may be stored with 1× Dilution Buffer with 0.02% sodium azide at 2–8 °C. **Do Not Freeze** the column.

Table 1.
Timetable for Rat 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	0.5	350
Wash	30.01	100	0	0	2.0	350
Elution	35.01	0	100	0	2.0	350
Neutralization	50.01	0	0	100	2.0	350
Re-equilibration	60.01	100	0	0	2.0	350
Stop	70.00					

Method for 12.7 × 79.0 mm column is optimized for Beckman System Gold HPLC, Pump Module 1 Type: 118, Detector Model: 166.

Appendix
Tips for fitting column to most chromatography instruments

1. Adapting the column (M6 fitting) to most HPLC systems
   - Option 1: female M6 to male 10-32 one-piece adapter (Catalog Number 55069) - Most HPLC instruments use 10-32 fittings with 1/16″ tubing, so an adapter (one-piece fitting) can be used to connect the column to the instrument. This inexpensive approach uses an adapter, a one-piece fitting with female M6 threading at one end, to accept the male M6 fitting on the tubing connected to the column, and 10-32 male threading on the other end, to fit into the detector or injector.
     Note: Use of this adapter will require removal of the fitting on the injector or detector.

   - Option 2: female M6/male 10-32 fitting to female 10-32/female 10-32 fitting two-piece adapter (Catalog Number 55068) – A two-piece adapter can be used without changing the detector or injector fittings on the HPLC. Again, the inexpensive approach is a two-piece adapter. The female M6 end of the female/male fitting accepts the male M6 fitting from the column, the male 10-32 end fits into one end of the female/ female 10-32 fitting. The other end of the female/female fitting accepts the 10-32 fitting on the HPLC tubing.

2. Adapting the column (M6 fitting) to non-metric medium/low pressure liquid chromatography systems
   - Option 1: female 1/4-28 to male 10-32 one-piece adapter (Catalog Number 55071) plus 10-32 to female M6 two-piece adapter (Catalog Number 55068) - Many medium/low pressure systems use 1/4-28 fittings. An one-piece adapter along with a two-piece adapter can be used to connect the column with M6 fittings to these instruments. This method requires having tubing with 1/4-28 fittings on the injector and detector. These fittings will thread into the female 1/4-28 to male 10-32 fitting (Catalog Number 55071). The male 10-32 will tread into the two-piece adapter (Catalog Number 55068) that will then join to the M6 fitting.

   - Option 2: female 1/4-28 to female M6 one-piece adapter (Catalog Number 59259-U) - Like Option 1, this option requires the tubing on the injector and detector already have male 1/4-28 fittings. The male 1/4-28 fitting will thread into the adapter that will then join it to the male M6 fitting.
     Note: Be sure to order two of these parts, one for each end of the column.

Troubleshooting Guide

Abnormal peak height – 60–70% 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.

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.

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