ProteoPrep® 20 Plasma Immunodepletion LC Column

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User Guide

Catalog Number

PROT20LC

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Ordering Information

Catalog No.	Product Description	Pkg Size
PROT20LC	ProteoPrep 20 Plasma Immunodepletion LC Column	1 each

Related Products

Catalog No.	Product Description	Pkg Size
PROT20	ProteoPrep 20 Plasma Immunodepletion Kit	1 Kit
PROT20S	ProteoPrep 20 Plasma Immunodepletion Kit Single	1 Kit
PROTIA	ProteoPrep Immunoaffinity Albumin and IgG Depletion Kit	1 Kit
PROTBA	ProteoPrep Blue Albumin and IgG Depletion Kit	1 Kit

To reorder product call 1-800-325-3010, visit our Web site at sigma-aldrich.com, or contact your local sales representative.

ProteoPrep® 20 Plasma Immunodepletion LC Column

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Product Description

The ProteoPrep 20 Plasma Immunodepletion LC Column is supplied complete with all necessary reagents and consumable equipment to deplete 20 high abundance proteins (listed below) from 100 µL of human plasma or serum. Accordingly, serum may be substituted wherever plasma is mentioned in the following procedures. Plasma may be depleted in preparation for proteomic analysis, two-dimensional electrophoresis (2DE), or liquid chromatography (LC). The ProteoPrep 20 Plasma Immunodepletion LC Column contains a mixture of affinity-purified polyclonal antibodies and small, single-chain antibody ligands bound to agarose. The ProteoPrep 20 Plasma Immunodepletion LC Column is a low pressure (less than 30 psi) column, and therefore, a low/medium pressure FPLC is highly recommended for its use.

Albumin (~45 mg/mL) and IgG (~10 mg/mL) are the two major protein components of human plasma, representing ~65% and 15% of total plasma proteins, respectively. The remaining plasma proteins depleted by this technology comprise a further 14–16% of the total human plasma protein.

Removal of these 20 high abundance proteins from human plasma (~95% of the total plasma protein content) allows for visualization of co-migrating proteins on an SDS-PAGE gel (either 1DE or 2DE) and also facilitates a higher sample load for improved visualization of lower copy number proteins. Specifically, this depletion technology facilitates a 20-fold increase in the relative amount of low abundance proteins.

A ProteoPrep 20 Plasma Immunodepletion LC column contains ~10 mL resin and will remove greater than 99% of albumin and IgG and greater than 95% of the remaining 18 abundant proteins detailed below, from a 100 µL plasma sample (40–50 mg/mL by Bradford Protein Assay) in a single pass, as determined by ELISA. With proper cleaning and storage, each column may be reused at least 100 times.

Note: Higher depletion levels can be expected if lower plasma volumes (e.g., 80 µL) are used. Individual plasma samples may vary in the concentration of the 20 high abundance proteins. Therefore, depletion levels may differ.

Depleted Proteins			
Albumin	Transferrin	α_1-Acid Glycoprotein	Complement C1q
IgG	Fibrinogen	Ceruloplasmin	Complement C3
IgA	α_2-Macroglobulin	Apolipoprotein A-I	Complement C4
IgM	α_1-Antitrypsin	Apolipoprotein A-II	Plasminogen
IgD	Haptoglobin	Apolipoprotein B	Prealbumin

Components	Catalog No.	Amount
ProteoPrep 20 LC Column	**P9874**	1 each
ProteoPrep 20 Equilibration Buffer, 10× concentrate — after dilution, the composition of the buffer is 1× phosphate buffered saline.	**P1749**	5 × 200 mL
ProteoPrep Elution Solution, MS Compatible, 10× concentrate — after dilution, the composition of the Elution Solution is 0.1 M Glycine-HCl, pH 2.5, and Octyl β-D-glucopyranoside	**P9749**	5 × 100 mL
ProteoPrep Preservative Concentrate	**K3889**	2 × 1.5 mL
Corning® Spin-X® Centrifuge Tube Filters	**CLS8160**	4 × 24 each
ProteoPrep LC Fittings	**F2805**	1 set

Reagents and Equipment Required But Not Provided

- Ultrapure water (Catalog No. W4502)
- Pipettors
- Low Pressure Liquid Chromatography Unit

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.

Storage and Stability

This kit is shipped on wet ice and components are stable for at least 2 years, as supplied, with proper storage at 2–8 °C.

The ProteoPrep 20 Equilibration Buffer, 10× concentrate (Catalog No. P1749) may precipitate at 2–8 °C. If precipitation occurs, allow the bottle to warm to room temperature and mix until completely dissolved prior to use.

Preparation Instructions

1× Equilibration Buffer (Buffer A)

Dilute one part of the ProteoPrep 20 Equilibration Buffer, 10× concentrate (Catalog No. P1749) with nine parts of ultrapure water in a clean bottle. Filter the diluted buffer through a 0.2 µm filter and degas for at least 5 minutes with sonication and vacuum. The final volume necessary for each depletion is 55 mL. Prepare more buffer than required in order to adequately fill and flush the FPLC system. Excess diluted 1× Equilibration Buffer should be discarded at the end of the day.

1× Elution Buffer (Buffer B)

Dilute one part of the ProteoPrep 20 Elution Solution (MS Compatible), 10× concentrate with nine parts of ultrapure water in a clean bottle. Filter the diluted buffer through a 0.2 µm filter and degas for 5 minutes with sonication and vacuum. The final volume necessary for each depletion is 30 mL. Prepare more buffer than required in order to adequately fill and flush the FPLC system. Excess diluted 1× Elution Buffer should be discarded at the end of the day.

Caution and Notes

- **Set the LC system maximum pressure at 30 psi. Higher pressures may compress the resin and reduce column efficiency and lifetime. A low/ medium pressure FPLC is recommended with this column. A high pressure liquid chromatography (HPLC) system is not recommended.**
- Alternative use of a high pressure liquid chromatography (HPLC) system (not recommended).
    - Most high pressure liquid chromatography (HPLC) systems require a certain amount of backpressure (e.g., greater than 100 psi) for the pumps to operate efficiently.
    - Necessary backpressure must be generated **in front of** the column (pump side) to prevent exposing the column to high pressures.
    - Backpressure may be generated using a backpressure regulator or by using 1–2 feet of small ID (0.005–0.007") PEEK tubing in front of the column.
    - Larger bore (0.030–0.040" ID) tubing must be used behind the column to prevent exposing the column to high pressures.

- When the column is received, small white spots may be visible in the column. These spots are due to the shipping (storage) buffer and will disappear after the column is equilibrated with the 1× Equilibration Buffer. The spots do not affect the performance of the column.
- If the LC system is also used for other applications, purge the system without the column attached with isopropanol and then with water for 15 minutes. Run one blank method with the 1× Equilibration and 1× Elution Buffers per the protocol prior to attaching the column to the system.
- **Do not expose the column to organic solvents (e.g., acetonitrile and alcohols), acids, bases, oxidizers, or reducing agents.**
- Recommended temperatures are indicated with each section.

Protocol (room temperature)

Note: The column should be run through the recommended program two times without a plasma sample when the column is received. The apolipoproteins are optimally depleted following 2 conditioning cycles.

1. FPLC setup and programming

a. Set the maximum pressure at 30 psi.

b. The following is a recommended FPLC program.

Recommended Program					
Step	Flow Rate (mL/min)	% Buffer A (equilibration)	% Buffer B (elution)	Step Volume (mL)	Step Time (min)
1	3.0	100	0	6.0	2.0
2	0.3	Inject Sample		0.1	< 1.0
3	0.3	100	0	15	50
4	3.0	0	100	30	10
5	3.0	100	0	30	10

c. Attach the supplied ferrules (inverted cone) and fitting nuts to the PEEK tubing of the chromatography unit. Screw the fitting nuts (and ferrules) into the supplied union.

Note: The cone end of the ferrules must be pointed toward the fitting nut so that the flat side of the ferrule will make contact with the column or supplied union.

2. Purge the lines first with 1× Elution Buffer (Buffer B) and then with 1× Equilibration Buffer (Buffer A).

3. Ensure that the column has come to room temperature before use.

4. **Caution — The order of this step is critical to preventing introduction of air into the column.**
    a. Stop the FPLC pumps and disconnect the fitting nuts (and ferrules) from the union.
    b. Only remove the gray plug from the top of the column. Start the pump at 0.5 mL/minute and drip 1× Equilibration Buffer into the red cap at the top of the column to fill. Stop the pump and screw the fitting nut (with ferrule and tubing) into the red cap.
    c. Next, remove the gray plug from the bottom of the column and screw the fitting nut in front of the UV detector into the red cap at the bottom of the column.

5. **Column Equilibration**
    a. First use, removal of the glycerol storage buffer — allow the column to equilibrate with 20–25 mL of 1× Equilibration Buffer (Buffer A) at 0.5 mL/minute. The pressure must not exceed 30 psi. If necessary, decrease the flow rate to lower the pressure.

    Note: The resin will change from opaque to white with the removal of glycerol.
    b. Subsequent uses — allow the column to equilibrate with 5 mL of 1× Equilibration Buffer (Buffer A) at 3 mL/minute. The pressure must not exceed 30 psi.

6. Filter undiluted plasma through the supplied Corning Spin-X Centrifuge Tube Filters (0.2 μm) at 14,000 × *g* for 5 minutes. Because a portion of the sample may be lost during filtering, be sure to filter 10–25% more sample than you intend to inject. (e.g., filter 125 μL for a 100 μL injection volume.)

7. Skip step 7 for the first 2 conditioning cycles uses of a new column. Inject 100 µL of the plasma (filtered) at a flow rate of 0.3 mL/minute. If the sample is injected through a sample loop, empty the loop with 1× Equilibration Buffer (Buffer A) using the total volume of the loop at the 0.3 mL/minute flow rate.

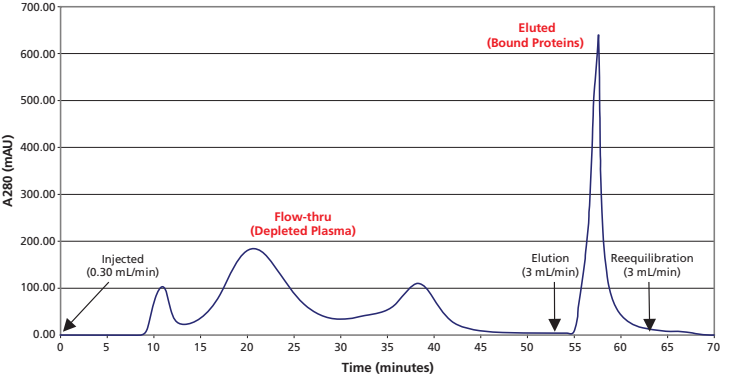
8. Wash the depleted plasma fraction from the column with 15 mL of 1X Equilibration Buffer (Buffer A) at 0.3 mL/minute. The depleted plasma fraction may be collected in 1 mL fractions or in bulk. The protein in the depleted plasma fraction typically elutes from the column from 9–43 minutes (3–13 mL).

   Note: Higher flow rates at this step may reduce efficiency of depletion.

9. Elute the bound proteins from the column with 30 mL of 1× Elution Buffer (Buffer B) at 1–3 mL/minute. The pressure must not exceed 30 psi.

10. Immediately reequilibrate the column with 30 mL of 1× Equilibration Buffer (Buffer A) at 1–3 mL/minute. The pressure must not exceed 30 psi.

Typical Depletion Chromatogram



Storage After Use

Short-term storage (less than 1 week)

1. The column must be equilibrated with a minimum of 15 mL of 1× Equilibration Buffer (5 minutes at 3 mL/minute).
2. Store the column in a refrigerator at 2–8 °C.
3. Make sure that the column does not experience temperatures below freezing.

Long-term storage (greater than 1 week)

1. Prepare a Preservative Equilibration Solution by adding 50 µL of ProteoPrep Preservative Concentrate to 25 mL of 1× Equilibration Buffer.
2. Equilibrate the column with a minimum of 15 mL of the Preservative Equilibration Solution (5 minutes at 3 mL/minute).
3. Store the column in a refrigerator at 2–8 °C. Make sure that the column does not experience temperatures below freezing.

Troubleshooting

- **High Backpressure** — If high backpressure is observed, first confirm that the pressure is not due to the chromatography system. For example, check that all system filters are clean and ensure that the sample has not clogged the small ID tubing. If the sample appears to be the source of high backpressure, it is possible that the anticoagulants are no longer effective and that the plasma is clotting. If this is the case, it is recommended that either EDTA or a protease inhibitor cocktail be added when the plasma sample is being diluted. This will inactivate proteases and reduce the activation of clotting cascades. (We suggest Protease Inhibitor Cocktail P1806. This product does not contain AEBSF or PMSF, which may complicate downstream analysis.)
- **Targeted Proteins Observed in Flow-through** — Given that the removal of targeted proteins is between 95–99%, the remaining protein may show activity in sensitive assays. In addition, diseased samples may have elevated levels of any number of the targeted proteins. If this is the case, decrease the amount of sample injected into the column.
- **Visible Head Space** — If a head space becomes visible at the top of the resin, the pump pressure has exceeded the recommended 30 psi. Stop the pumps and allow the resin to swell back to original volume.

Quick Reference Protocol

Program FPLC and attach supplied fittings

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Purge lines with Elution Buffer and then Equilibration Buffer
Filtered and degassed buffers

↓

Attach the PROT20LC column

↓

Equilibrate the column
First use – 15 mL 1× Equilibration Buffer (0.5 mL/minute)
Subsequent uses – 5 mL 1× Equilibration Buffer (3 mL/minute)

↓

Inject 100 µL of filtered plasma
0.3 mL/minute
Skip this step for the first 2 conditioning uses of a new column.

↓

Wash depleted plasma from column
15 mL 1× Equilibration Buffer (0.3 mL/minute)

↓

Elute bound proteins from column
30 mL 1× Elution Buffer (3 mL/minute)

↓

Reequilibrate the column
30 mL 1× Equilibration Buffer (3 mL/minute)

Important Reminder

The Equilibration and Elution Buffers must be at room temperature, filtered and degassed prior to use with the column to prevent gas bubble generation in the column. The column must also be allowed to warm to room temperature before use.

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