User Guide

Catalog Nos.
PROT20
PROT20S

ProteoPrep® 20 Plasma Immunodepletion Kit

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Ordering Information

Catalog No.	Product Description	Pkg Size
PROT20	ProteoPrep 20 Plasma Immunodepletion Kit	1 Kit
PROT20S	ProteoPrep 20 Plasma Immunodepletion Kit Single	1 Kit

Related Products

Catalog No.	Product Description	Pkg Size
PROTBA	ProteoPrep Blue Albumin and IgG Depletion Kit	1 Kit
PROTIA	ProteoPrep Immunoaffinity Albumin and IgG Depletion Kit	1 Kit
PROT20LC	ProteoPrep20 Plasma Immunodepletion LC Column	1 Each

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 Kit

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Product Description

The ProteoPrep 20 Plasma Immunodepletion Kit is a complete kit with all necessary reagents and consumable equipment to deplete 20 highly abundant proteins from human plasma or serum. Accordingly, serum may be substituted wherever plasma is mentioned in the following procedures. This kit is designed to specifically remove the 20 proteins from human plasma listed in the table below. Specifically, 8 µL of plasma may be depleted in preparation for proteomic analysis, two-dimensional electrophoresis (2DE), or liquid chromatography (LC). The ProteoPrep 20 Plasma Immunodepletion medium contains a mixture of affinity-purified polyclonal IgGs and small single-chain antibody ligands attached to agarose, and is prepacked in a spin column.

Albumin (~45 mg/mL) and IgG (~10 mg/mL) are the two major protein components of human plasma, representing approximately 65% and 15% of total plasma proteins, respectively.[1] The remaining plasma proteins depleted by this technology comprise a further 17–19% of the total human plasma protein.

Removal of these "top 20" proteins from human plasma (~97% 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 30 to 50-fold increase in the relative amount of low abundance proteins.

A ProteoPrep 20 spin column will remove ~ 99% of the 20 highly abundant proteins detailed below, from an 8 µL plasma sample (40–50 mg/mL by Bradford Protein Assay), as determined by ELISA. With proper cleaning and storage, each column may be reused at least 100 times.

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.	Amt/No. in Kit PROT20	PROT20S
ProteoPrep 20 Plasma Immunodepletion Columns — supplied as prepacked spin columns with 300 µL of packed medium. The medium is stored in phosphate buffered saline with 50% (v/v) glycerol and 0.0015% (w/v) Kathon® CG/ICPII, an antimicrobial preservative.	**P2249**	3	1
ProteoPrep 20 Equilibration Buffer, 10× concentrate — after dilution, the composition of the buffer is 1× phosphate buffered saline.	**P1749**	200 mL	200 mL
ProteoPrep 20 Elution Solution, 10× concentrate — after dilution, the composition of the Elution Solution is 0.1 M Glycine-HCl, pH 2.5, and TWEEN® 20.	**P1624**	100 mL	100 mL
ProteoPrep Preservative Concentrate	**K3889**	1.5 mL	1.5 mL
Luer Lock Caps	**L1543**	6	6
Luer Lock Syringes	**Z248010**	6	6
Corning® Spin-X® Centrifuge Tube Filters	**CLS8160**	2 × 24	1 × 24
Vivaspin 500 Centrifugal Concentrators — pore size 5,000 Da MWCO	**Z614009**	25	25
Collection Tubes, 2 mL	**T5449**	50	50

Reagents and Equipment Required But Not Provided

- Ultrapure water (**Cat. No. W4502**)
- Micropipettors
- Disposable plastic tubes (5–50 mL capacity)
- Microcentrifuge (capable of operating at 5000 rpm)

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 1 year, 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

Dilute one part of the ProteoPrep 20 Equilibration Buffer, 10× concentrate (Catalog No. P1749) with nine parts of ultrapure water in a clean tube. The final volume necessary for each plasma application is 5 mL. Excess diluted Equilibration Buffer should be discarded at the end of the day.

1× Elution Solution

Dilute one part of the ProteoPrep 20 Elution Solution, 10× concentrate (Catalog No. P1624) with nine parts of ultrapure water in a clean tube. The final volume necessary for each plasma application is 2 mL. Excess diluted Elution Solution should be discarded at the end of the day.

Procedure

Recommended temperatures are indicated with each section.

A. Column Equilibration (room temperature)

1. Remove the bottom plug, loosen upper screw cap from the spin column, and place in a 2 mL Collection Tube (Catalog No. T5449).
2. Centrifuge the spin column and collection tube at 1000–2000 × *g* (4000–5000 rpm for most rotors) for 30–60 seconds.
3. Remove the screw cap and attach a Luer Lock Cap (Catalog No. L1543) onto the column. Draw 4 mL of 1× Equilibration Buffer into a Luer Lock Syringe (Catalog No. Z248010) and attach the syringe to the Luer Lock Cap. Slowly push the 1× Equilibration Buffer through the resin into a disposable tube capable of holding at least 5 mL.
4. (Optional) If buffer exchange or dialysis will be used on the sample after depletion, it is recommended that step 3 (above) be repeated to remove remaining traces of TWEEN 20 left from previous elution(s) of bound proteins. For samples that will be precipitated (e.g., TCA, acetone, ethanol), following depletion, an extra
4 mL of 1× Equilibrium Buffer is unnecessary.
5. Remove the syringe and Luer Lock cap from the column and place the column in a collection tube. Loosely place the screw cap on the column with a half turn. Centrifuge the spin column for 30 seconds at 1000–2000 × *g* (4000–5000 rpm for most rotors). Discard the buffer in the collection tube and place the spin column into a fresh 2 mL collection tube.

B. Plasma/Serum Depletion (room temperature)

Depletion of 8 µL of plasma (or serum) will typically remove 90–95% of each of the 20 highly abundant proteins during the initial depletion and >99% when the plasma sample is redepleted (passed through the resin a second time). Should it prove necessary to pool the depleted plasma from several aliquots of plasma, it is recommended that the pool be concentrated and then passed through the column a final time. For details, see sections D and E on pages 8 and 9.

1. Dilute a plasma sample (typically 8 µL) to 100 µL with 1× Equilibration Buffer and filter (0.2 µm) through a Corning Spin-X Centrifuge Tube Filter (Catalog No. CLS8160). Centrifuge as much as 500 µL of diluted plasma at 1000–2000 × *g* (4000–5000 rpm for most rotors) for 30–60 seconds. If more than 500 µL of diluted plasma is prepared, the filter may be reused.
2. Add 100 µL of diluted and filtered plasma to the top of the packed bed of medium. The sample will immediately absorb into the medium, ensuring efficient binding and minimal sample dilution. Incubate at room temperature for 15–20 minutes.
3. Centrifuge the spin column and collection tube at 1000–2000 × *g* (4000–5000 rpm for most rotors) for 30–60 seconds. Save the flow-through (depleted plasma) in the tube.
4. Wash the remaining depleted plasma proteins from the spin column by adding 100 µL of 1× Equilibration Buffer to the top of the medium bed and centrifuge at 1000–2000 × *g* (4000–5000 rpm for most rotors) for 30–60 seconds. Collect the wash in the same tube.
5. Repeat this wash step with an additional 100 µL of 1× Equilibration Buffer. The majority (>95%) of unbound proteins will be in this pool of depleted plasma (0.3 mL).
6. For long-term storage, store the depleted plasma at or below –20 °C.

   Note: Depletion levels above 99% require a second depletion (see section E).

C. Elution of Bound Proteins (room temperature)

A minor number of proteins, besides those specifically depleted, may bind to the depleted proteins. These bound proteins may also be analyzed to confirm that the protein(s) of interest are not bound.

1. To elute the bound proteins, screw a Luer Lock Cap onto the column.
2. Draw 2 mL of 1× Elution Solution into a syringe and attach the syringe to the Luer Lock Cap. Slowly push the 1× Elution Solution through the resin into a disposable tube capable of holding at least 5 mL. Note that this step should take ~1 minute (~1 drop/second). For analysis of the eluted protein solution, neutralize by adding a volume of 1 M Trizma® Base solution equal to 0.05 volume of the eluted material (0.1 mL of 1 M Trizma base for 2 mL of eluted protein).
3. The bound protein extract may be stored at or below –20 °C. To remove the glycine and TWEEN 20, acetone precipitation is recommended. See Section G on page 10 for the details of a suggested acetone precipitation protocol.
4. The spin column should be immediately re-equilibrated to reduce exposure time of the resin to acidic conditions. Re-equilibration is performed by completing steps A.3 to A.5.

D. Concentration of Multiple Depletions (2–8 °C)

Pooling and concentrating multiple depletions (up to 10) from the same plasma sample may be necessary to obtain sufficient quantities for detection of lower abundance proteins. Two microcentrifuge filters should be used for each set of 10 depletion cycles.

1. Add 0.2 mL of high purity water to 2 Vivaspin 500 Centrifugal Concentrators (Catalog No. Z614009) and microcentrifuge at 5000 × *g* (7000–8000 rpm for most rotors) for 15 minutes in a cold room. This step will remove any extractables from the filter.
2. Remove the water from the tube and filters by shaking. Do not contact the membrane with a pipette tip until step 4.
3. Following each depletion, add the depleted plasma to the filters (0.15 mL per filter) and centrifuge at 5000 × *g* for 20–30 minutes in a cold room. Continue adding the depleted plasma to the same filter until the desired number of depletions have been carried out.
4. The final volume of the pooled and concentrated depleted plasma should be 0.1–0.2 mL. Transfer the concentrate to a separate microcentrifuge tube. Add 0.1 mL of 1× Equilibration Buffer to the filter, pipette up and down around the filter surface, and pool this wash with the concentrate.

E. Final Depletion (room temperature)

A final depletion of the pooled and concentrated depleted plasma (from up to 10 depletion cycles) is recommended and will raise the average level of depletion to >99%.

1. Carry out a final depletion on the concentrated depleted plasma (0.2–0.3 mL from step D.4) by adding 0.1 mL to the re-equilibrated column (step A.5). Incubate for 20 minutes and centrifuge at 1000–2000 × *g* (4000–5000 rpm for most rotors) for 30–60 seconds.
2. If necessary, add another 0.1 mL of concentrated depleted plasma to the column and incubate for 20 minutes and centrifuge as above.
3. After all the concentrated depleted plasma has been added to the resin column and centrifuged, wash the column two times with 0.1 mL of 1× Equilibration Buffer (see steps B.4 and B.5). Pool these washes with the twice-depleted concentrated depleted plasma.
4. Elute the bound proteins from the column into a disposable tube capable of holding at least 5 mL (see steps C.1 to C.4) and re-equilibrate the column (see steps A.3 to A.5).
5. The depleted sample may now be analyzed directly or further precipitated.

F. Column Storage (room temperature and 2–8 °C)

Important Reminder

*The column plug **must** be inserted only after centrifuging the 1× Equilibration Buffer from the spin column. Plugging the column without centrifugation could lead to the frit being pushed up by hydraulic pressure.*

1. Short-term storage (less than 1 week)

   Immediately wash the resin by drawing 4 mL of 1× Equilibration Buffer into a syringe and attach the syringe to the Luer Lock Cap. Slowly push the 1× Equilibration Buffer through the resin into a disposable tube capable of holding at least 5 mL. **Centrifuge the spin column and collection tube at 1000–2000 × *g* (4000–5000 rpm for most rotors) for 30–60 seconds.** Push in the bottom plug and then add 0.3 mL of 1× Equilibration Buffer to the top of the resin. Screw the top cap onto the column and store the column at 2–8 °C.

2. For longer-term storage (greater than 1 week)

   Add 10 µL of ProteoPrep Preservative Concentrate to 5 mL of 1× Equilibration Buffer and follow the protocol for short-term storage described above.

G. Acetone Precipitation (2–8 °C and –20 °C)

1. Add one volume of the eluted bound protein fraction (2 mL) to a centrifuge tube (acetone safe) capable of holding a total volume of 6× the volume of the eluted bound protein fraction.
2. Add a volume of cold (–20 °C) 100% acetone equal to 5× the volume of the eluted bound protein fraction. Store the tube at –20 °C overnight.
3. Centrifuge at 15,000 × *g* for 30 minutes at 2–8 °C.
4. Remove the supernatant (decant) and suspend the pellets by vortexing in cold (–20 °C) 50% acetone equal to 5× the volume of the eluted bound fraction.
5. Centrifuge at 15,000 × *g* for 30 minutes at 2–8 °C.
6. Remove the supernatant (decant) and resuspend the pellets with cold (–20 °C) 50% acetone equal to 5× the volume of the eluted bound fraction.
7. Centrifuge the tubes at 15,000 × *g* for 30 minutes at 2–8 °C and remove the supernatant (decant).
8. Allow the pellet to air dry at room temperature (preferably overnight).
9. Dissolve the protein pellet in an appropriate reagent.

Other Related Products

- Tributylphosphine Solution (**T7567**) and Iodoacetamide (**A3221**)
  or
  ProteoPrep Reduction and Alkylation Kit (**PROTRA**)
- ProteoPrep Protein Precipitation Kit (**PROTPR**)
- Laemmli 2× Sample Buffer (**S3401**)
- EZBlue™ Gel Staining Reagent (**G1041**)
- ProteoSilver™ Plus Silver Staining Kit (**PROTSIL2**)
- SYPRO® Ruby Protein Gel Stain (**S4942**)
- ProteoGel™ IPG Strip, pH 4–7:
  - 7 cm (**I2906**)
  - 11 cm (**I3531**)
  - 18 cm (**I4156**)

References

1. Rengarajan, K. et al., Removal of albumin from multiple human plasma samples. *BioTechniques,* **1996,** *20,* 30.

Notes

Quick Reference Protocol

Sample Preparation
Dilute 8 µL of plasma and filter

↓

Prepare Spin Column

↓ *Centrifuge for 5 sec*

Column Equilibration
4 mL of 1× Equilibration Buffer

↓ *Centrifuge for 30 sec*

Add Sample to Column
100 µL

↓ *Incubate for 20 min, Centrifuge for 60 sec*

Column Wash
1× Equilibration Buffer (2 × 100 µL)

↓ *Centrifuge for 60 sec*

Depleted Plasma
300 µL, ~95% depletion

↓

Concentrate Depleted Plasma
Up to 10 depletions to 300 µL

↓

Add Sample to Column
100 µL at a time

↓ *Incubate for 20 min, Centrifuge for 60 sec*

Column Wash
1× Equilibration Buffer (2 × 100 µL)

↓ *Centrifuge for 60 sec*

Depleted Plasma
500 µL, >99% depletion

Elute Bound Proteins
2 mL of 1× Elution Buffer

↓ *Neutralize*

Eluted Proteins

Important Reminder

*For column storage, the column plug **must** be inserted only after centrifuging the 1× Equilibration Buffer from the spin column. Plugging the column without centrifugation could lead to the frit being pushed up by hydraulic pressure.*
(See page 10, Important Reminder.)

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