



## Ordering Information

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

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# 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 high abundance 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  $\mu$ L 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.<sup>1</sup> 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 approximately 99% of the 20 abundant proteins detailed below, from an 8  $\mu$ L plasma sample (50–70 mg/mL), as determined by ELISA. With proper cleaning and storage, each column may be reused at least 100 times.

Depleted Proteins	
Albumin	$\alpha_1$ -Acid Glycoprotein
IgG	Ceruloplasmin
IgA	Apolipoprotein A-I
IgM	Apolipoprotein A-II
IgD	Apolipoprotein B
Transferrin	Complement C1q
Fibrinogen	Complement C3
$\alpha_2$ -Macroglobulin	Complement C4
$\alpha_1$ -Antitrypsin	Plasminogen
Haptoglobin	Prealbumin

<b>Components</b>	<b>Catalog No.</b>	<b>Amt/No. in Kit</b>
ProteoPrep 20 Plasma Immunodepletion Columns — supplied as prepacked spin columns with 300 $\mu$ L 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.	<b>P2249</b>	3 each
ProteoPrep 20 Equilibration Buffer, 10 $\times$ concentrate — after dilution, the composition of the buffer is 1 $\times$ phosphate buffered saline.	<b>P1749</b>	200 mL
ProteoPrep 20 Elution Solution, 10 $\times$ concentrate — after dilution, the composition of the Elution Solution is 0.1 M Glycine-HCl, pH 2.5, and TWEEN® 20.	<b>P1624</b>	100 mL
ProteoPrep Preservative Concentrate	<b>K3889</b>	1.5 mL
Luer Lock Caps	<b>L1543</b>	6
Luer Lock Syringes	<b>Z719900</b>	6
Corning® Spin-X® Centrifuge Tube Filters	<b>CLS8160</b>	2 $\times$ 24
Ultrafree®-MC Microcentrifuge Filters, NMWL 5000 Da	<b>M0286</b>	25
Collection Tubes, 2 mL	<b>T5449</b>	50

## Reagents and Equipment Required But Not Provided

- Ultrapure water (Catalog 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.

## Preparation Instructions

**Equilibration Buffer** Dilute one part of the ProteoPrep 20 Equilibration Buffer 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.

**Elution Solution** Dilute one part of the ProteoPrep 20 Elution Solution 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.

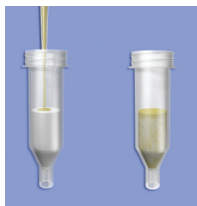
### A. Column Equilibration (room temperature)



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  $1000\text{--}2000 \times g$  (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 prepared Equilibration Buffer into a Luer Lock Syringe (Catalog No. Z719900) and attach the syringe to the Luer Lock Cap. Slowly push the Equilibration Buffer through the resin into a disposable tube capable of holding at least 5 mL.
4. 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  $1000\text{--}2000 \times g$  (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  $\mu\text{L}$  of plasma (or serum) will typically remove 90–95% of each of the 20 high abundance 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  $\mu\text{L}$ ) to 100  $\mu\text{L}$  with the Equilibration Buffer and filter (0.2  $\mu\text{m}$ ) through a Corning Spin-X Centrifuge Tube Filter (Catalog No. CLS8160). Centrifuge as much as 500  $\mu\text{L}$  of diluted plasma at  $1000\text{--}2000 \times g$  (4000–5000 rpm for most rotors) for 30–60 seconds. If more than 500  $\mu\text{L}$  of diluted plasma is prepared, the filter may be reused.
2. Add 100  $\mu\text{L}$  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  $1000\text{--}2000 \times g$  (4000–5000 rpm for most rotors) for 30–60 seconds. Save the flow-through (depleted plasma) in the tube.
4. Wash the remaining unbound proteins from the spin column by adding 100  $\mu\text{L}$  of Equilibration Buffer to the top of the medium bed and centrifuge at  $1000\text{--}2000 \times g$  (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  $\mu\text{L}$  of 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  $-20^\circ\text{C}$ .



### **C. Elution of Bound Proteins (room temperature)**

A minor number of proteins, besides those specifically depleted, may bind to the depleted proteins or medium. 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 prepared Elution Solution into a syringe and attach the syringe to the Luer Lock Cap. Slowly push the Elution Solution through the resin into a disposable tube capable of holding at least 5 mL. Note that this step should take ~1 minute. 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 an 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 and A.4.

#### **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 water to 2 Ultrafree-MC microcentrifuge filters (Catalog No. M0286) and microcentrifuge at  $5000 \times g$  (7000–8000 rpm for most rotors) for 15 minutes in a cold room. This step will remove any extractables from the membrane.
2. Remove the water from the tube and filters.
3. Following each depletion, add the depleted plasma to the filters (0.15 mL per filter) and centrifuge at  $5000 \times g$  for 20–30 minutes in the 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 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.4). Incubate for 20 minutes and centrifuge at  $1000\text{--}2000 \times g$  (4000–5000 rpm for most rotors) for 30–60 seconds.
- 2.** 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 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 and A.4).
- 5.** The depleted sample may now be analyzed directly or further precipitated.

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

The column plug should only be inserted after centrifuging the 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 prepared Equilibration Buffer into a syringe and attach the syringe to the Luer Lock Cap. Slowly push the Equilibration Buffer through the resin into a disposable tube capable of holding at least 5 mL. **Centrifuge the spin column and collection tube at  $1000\text{--}2000 \times g$  ( $4000\text{--}5000 \text{ rpm}$  for most rotors) for 30–60 seconds.** Push in the bottom plug and then add 0.3 mL of Equilibration Buffer to 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  $\mu\text{L}$  of ProteoPrep Preservative Concentrate to 5 mL of 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  $6\times$  the volume of the eluted bound protein fraction.
2. Add a volume of cold (–20 °C) 100% acetone equal to  $5\times$  the volume of the eluted bound protein fraction. Store the tube at –20 °C overnight.
3. Centrifuge at  $15,000 \times g$  for 30 minutes at 4 °C.
4. Remove the supernatant (decant) and suspend the pellets by vortexing in cold (–20 °C) 50% acetone equal to  $5\times$  the volume of the eluted bound fraction.
5. Centrifuge at  $15,000 \times g$  for 30 minutes at 4 °C.

6. Remove the supernatant (decant) and resuspend the pellets with cold ( $-20^{\circ}\text{C}$ ) 50% acetone equal to  $5\times$  the volume of the eluted bound fraction.
7. Centrifuge the tubes at  $15,000 \times g$  for 30 minutes at  $4^{\circ}\text{C}$  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

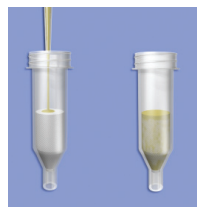
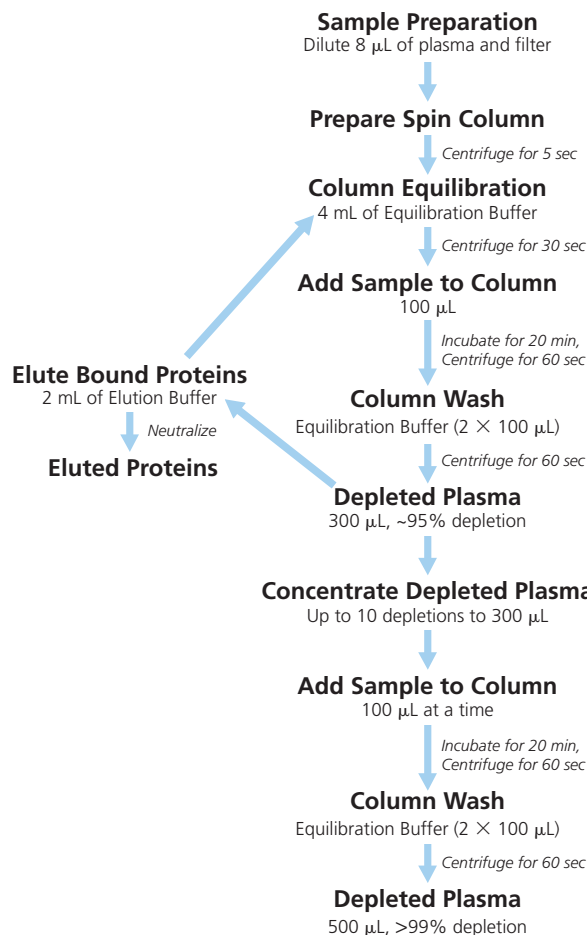
- Tributylphosphine Solution (**T7567**) and Iodoacetamide (**A3221**)  
**or**  
ProteoPrep Reduction and Alkylation Kit (**PROTRA**)
- ProteoPrep Protein Precipitation Kit (**PROTPR**)
- Laemmli  $2\times$  Sample Buffer (**S3401**)
- EZBlue™ Gel Staining Reagent (**G1041**)
- ProteoSilver™ Plus Silver 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

## Experienced User Protocol



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