Product Information

ProteoPrep® Immunoaffinity Albumin and IgG Depletion Kit

For 2D Electrophoresis and Liquid Chromatography

PROTIA

Storage temperature: 2-8 °C

Product Description

The ProteoPrep® Immunoaffinity Albumin and IgG Depletion Kit has been designed to specifically remove albumin and IgG from human serum (25–50 µL) in preparation of samples for proteomics analysis, two-dimensional electrophoresis (2DE) or liquid chromatography (LC). The ProteoPrep® Immunoaffinity medium in the prepacked spin columns is a mixture of two beaded mediums containing recombinantly expressed, small single-chain antibody ligands, resulting in low non-specific binding and high capacity. This kit is targeted toward human albumin and IgG and will demonstrate a slight decrease in capacity for depletion of mouse serum. Albumin (~45 mg/mL) and IgG (~10 mg/mL) are the two major protein components of human serum, representing approximately 65% and 15% of the total serum proteins, respectively.[1] Removal of albumin and IgG from serum allows the visualization of co-migrating proteins on an SDS PAGE gel (1DE or 2DE) and also allows a higher sample load (4 to 5-fold) for improved visualization of lower copy number proteins. This kit is designed to use a serum volume of 50 mL per each of the ProteoPrep® Immunoaffinity Columns. A spin column of medium will remove greater than 95% of the albumin and 85% of the IgG from a 50 µL serum sample as determined by ELISA. A greater depletion of albumin and IgG can be expected with a smaller serum sample (10 to 45 µL), due to a lower sample to medium ratio.

Components	Catalog Number	PROTIA
ProteoPrep® Immunoaffinity Columns Supplied as prepacked spin columns with 350 µL of packed medium. The medium is equilibrated in phosphate buffered saline with 50% (v/v) glycerol and 0.0015% (w/v) Kathon® CG/ICPII, an antimicrobial preservative.	P4250	10 each
ProteoPrep® Immunoaffinity Equilibration Buffer Supplied as a tablet (in a bottle) that reconstitutes to a final volume of 30 mL of a low ionic strength Tris buffer, pH 7.4.	P4121	1 each
Protein Extraction Reagent Type 4 Supplied as a powder that upon reconstitution provides 23 mL of 40 mM Trizma® Base, 7.0 M urea, 2.0 M thiourea, and 1% C7BzO detergent, pH 10.4.	C0356	1 each
Collection Tubes, 2 mL	T5449	3 x 10 each

Reagents and Equipment Required (Not Provided)

- High purity water (W4502)
- 30 °C water bath
- Micropipettors
- Graduated cylinder
- Microcentrifuge (to 10,000 rpm)

Precautions and Disclaimer

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

Preparation Instructions

Equilibration Buffer

Add 30 mL of high purity water to the bottle of ProteoPrep® Immunoaffinity Equilibration Buffer (P4121). The final volume is 30 mL. The Equilibration Buffer can be stored at 4 °C for up to 2 weeks or it may be stored in aliquots at –20 °C for longer term storage.

Protein Extraction Reagent Type 4

It is advisable to read the entire Procedure to determine the form and amount of this reagent required for serum sample preparation. If this reagent is used for 2DE sample preparation (Procedure, Two-Dimensional Electrophoresis Sample Preparation), it may be advisable to dissolve the sample in the powdered reagent, especially if large volumes of a dilute sample are used. To prepare 0.3 mL of the liquid reagent, weigh out 178 mg of the powdered reagent and add 0.2 mL of high purity water. Alternatively, the entire contents of the bottle may be dissolved. Adding 15 mL of high purity water to the contents of the Protein Extraction Reagent Type 4 bottle yields a final volume of 23 mL.

Note: The solution will initially become cold to the touch. Warm the bottle for ~30 minutes while mixing periodically to ensure the powder is completely dissolved. A 30 °C water bath will aid in dissolving the powder. Do not allow the temperature of the solution to rise above 30 °C because cyanates detrimental to the proteins may form. Aliquot the unused reagent in 1–2 mL volumes, and freeze at –20 °C. The frozen reagent is stable for 6 months.

Storage and Stability

This kit ships on wet ice and the components are stable for at least 1 year, as supplied, with proper storage at 2–8 °C. The Collection Tubes, ProteoPrep® Immunoaffinity Equilibration Buffer and the Protein Extraction Reagent Type 4 may be stored at room temperature upon receipt.

Procedure

This procedure has been optimized for use at room temperature (15–25 °C).

Column Equilibration

1. Break off the bottom tip of the spin column(s) and loosen the red cap approximately 1 full turn. Centrifuge the spin column(s) in a 2 mL collection tube (tube cap toward center of rotor) at 8,000 rpm (5,000 x *g*) for 5–10 seconds to remove the storage solution. Because of the height of the columns, it may be necessary to use alternating positions in the rotor. Discard the buffer in the collection tube.

   Note: Do not use the pulse button on the microcentrifuge. This may bypass the speed control and centrifuge at a higher speed. Higher speeds may compromise the column frit and allow the medium to leak through.
2. Add 0.4 mL of the Equilibration Buffer to the medium in the spin column and centrifuge (5,000 x *g*) for 5–10 seconds. Discard the buffer in the collection tube and place the spin column back into the same collection tube.
3. Repeat the equilibration step (step 2) two times. After the final equilibration, centrifuge each spin column for 30 seconds. Discard the buffer in the collection tube and place the spin column into a fresh collection tube. Save the equilibration collection tube for the optional elution of bound proteins procedure (Elution of Bound Proteins, step 1).

Serum/Plasma Depletion of Albumin and IgG

1. Dilute a serum/plasma sample (typically 25–50 µL) to 100 µL with the Equilibration Buffer and add to the top of the packed medium bed. The sample will immediately adsorb into the medium ensuring efficient binding and minimal sample dilution. Incubate at room temperature for 5–10 minutes.
2. Centrifuge the spin column and collection tube at 10,000 rpm (8,000 *g*) for 60 seconds.
3. Reapply the eluate in the collection tube to the top of the medium bed. Incubate for 5–10 minutes at room temperature. This step will ensure optimal depletion.
4. Centrifuge the spin column in the same collection tube as before for 60 seconds. The twice depleted serum should remain in the collection tube and will be combined with the wash from step 5 for optimal protein recovery.
5. Wash the remaining unbound proteins from the spin column by adding 125 µL of the Equilibration Buffer to the top of the medium bed and centrifuge for 60 seconds. Collect the wash in the same tube used in step 4. The majority (> 95%) of the unbound proteins will be in the depleted serum/plasma sample.
6. Store the albumin/IgG depleted serum/plasma at or below –20 °C for long term storage.

Elution of Bound Proteins (Optional)

A minor number of proteins, besides albumin and IgG, may bind to the medium. It is recommended that these bound proteins also be analyzed the first time that the ProteoPrep® Immunoaffinity Albumin and IgG Depletion Kit is used to ensure that the protein(s) of interest are not bound.

The bound proteins extracted with the prepared Protein Extraction Reagent Type 4 are both SDS-PAGE and 2DE compatible. For SDS-PAGE, steps 3 and 4 may be performed using 2X Laemmli Sample Buffer (S3401, not supplied) in place of the Protein Extraction Reagent Type 4.

1. Wash the remaining trace amounts of unbound protein from the column by placing the spin column in the collection tube used for column equilibration (Column equilibration, step 3). Add 0.4 mL of the ProteoPrep® Immunoaffinity Equilibration Buffer to the top of the packed medium and centrifuge at 10,000 rpm (8,000 x *g*) for 60 seconds. Repeat this wash step, (once only).
2. To elute the bound proteins, transfer the column to a new collection tube.
3. Add 150 µL of the prepared Protein Extraction Reagent Type 4 to the top of the packed medium and centrifuge at 10,000 rpm (8,000 x *g*) for 60 seconds. The eluant should remain in the collection tube and will be combined with the eluant from the next step.
4. Add a second 150 µL volume of Protein Extraction Reagent Type 4 to the top of the packed medium and centrifuge at 10,000 rpm (8,000 x *g*) for 60 seconds, collecting the eluant in the same tube used in step 3. The combined 300 µL extract contains greater than 95% of the bound proteins.
5. It is recommended to immediately dilute an aliquot of the bound protein extract for either SDS-PAGE or 2DE and then store the diluted sample at or below –20 °C. The remainder of the bound protein extract should be stored at 2–8 °C, because freezing the concentrated bound protein extract may cause proteins (for example, albumin) to precipitate.

Two-Dimensional Electrophoresis Sample Preparation

1. The appropriate sample volume will be based on the gel staining procedure to be used (for example, Coomassie, silver, or fluorescent staining). For a Coomassie stained gel, a portion of the depleted serum (Serum/Plasma Depletion of Albumin and IgG, step 6) corresponding to 5–40 µL of the original serum is recommended. For silver or fluorescent staining, a portion of the depleted serum corresponding to 0.5–4 µL of original serum is recommended.
2. Dilute a sample of the depleted serum (or bound protein extract) with the Protein Extraction Reagent Type 4 to the volume recommended by the strip manufacturer for rehydration of the IPG strip (typically 125 µL for 7 cm strips, 200 µL for 11 cm strips, and 300 µL for 18 cm strips).

   Note: If the depleted serum sample is greater than 25% of the desired final volume for rehydration of the IPG strip, it is recommended that the Protein Extract Reagent Type 4 be used in the powdered form. For a 300 µL IPG strip rehydration volume, dilute the depleted serum sample to 200 µL with high purity water and add 178 mg of powdered Protein Extraction Reagent Type 4.
3. It is recommended the sample be reduced and alkylated prior to running the first dimension (IPG strip). This step can be performed using the ProteoPrep® Reduction and Alkylation Kit (PROTRA). Alternatively, the following method may be used: For reduction with tributylphosphine (TBP, T7567), add 1 µL of the 0.2 M TBP solution for every 40 µL of diluted sample and incubate at room temperature for 30–60 minutes. Following reduction, alkylate with iodoacetamide at a concentration of 15 mM. Add 1 µL of a 0.5 M iodoacetamide solution prepared with A3221 for every 33 µL of diluted sample and incubate at room temperature for 30–60 minutes.
4. After reduction and alkylation, the sample is ready for IPG strip rehydration. For serum samples, IPG strips with pH ranges of 3–10 or 4–7 are recommended. Majority of the proteins will focus within the pH range of 4–7.

Other Related Products

- Tributylphosphine (T7567) and Iodoacetamide (A3221) or ProteoPrep® Reduction and Alkylation Kit (PROTRA)
- Laemmli 2X Sample Buffer (S3401)
- EZBlue™ Gel Staining Solution (G1041)
- ProteoSilver™ Plus Staining Kit (PROTSIL2)
- SYPRO® Ruby (S4942)

Troubleshooting Guide

Problem	Possible Cause	Solution
Poor albumin and/or IgG depletion	Large serum load	Decrease the serum load.
	Insufficient incubation time of serum with medium	Allow the serum to incubate on the medium for at least 5 minutes.
High non-specific binding	Low serum load	Increase the amount of serum loaded on the High non-specific binding medium.
	Inadequate medium equilibration	Centrifuge the medium and then equilibrate it at least two times.

References

1. Rengarajan, K. et al., Removal of albumin from multiple human serum samples. BioTechniques, 20, 30-32 (1996).

Notice

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