SIGMA-ALDRICH®

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Product Information

ProteoPrep® Blue Albumin and IgG Depletion Kit
For 2D Electrophoresis

Product Number **PROTBA**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

The ProteoPrep® Blue Albumin and IgG Depletion Kit has been designed to specifically remove albumin and IgG from 25 samples of human serum (25–100 µl) in preparation for two-dimensional (2D) electrophoresis. The ProteoPrep Blue Albumin and IgG Depletion Medium is a mixture of two medias: 1) a blue dye conjugated to an agarose base matrix and 2) Protein G agarose.

Albumin (~45 mg/ml) and IgG (~10 mg/ml) are two major protein components of serum, representing 60–70% and 10–20 % of the total serum protein, respectively.[1] Removal of albumin and IgG from serum allows the visualization of co-migrating proteins on a 1D or 2D electrophoresis gel and also allows a higher sample load (4 to 5-fold) for improved visualization of lower copy number proteins.

Components

- ProteoPrep Blue Albumin and IgG Depletion Medium (Product Number P1120), supplied as a 10 ml of suspension containing 60% packed medium.
- ProteoPrep Blue Equilibration Buffer (Product Number P1245), supplied as a powder that reconstitutes to a final volume of 50 ml of a low ionic strength, Tris-buffered urea solution, pH 7.8.
- Protein Extraction Reagent Type 4 (Product Number C0356), 1 bottle of powder that reconstitutes to a final volume of 23 ml of a solution containing 7.0 M urea, 2.0 M thiourea, 1 % C7BzO detergent, and 40 mM Trizma®, pH 10.4.
- 25 Spin Columns, Product Number HP6787
- 100 Collection Tubes, 2 ml, Product Number T5449

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.

Reagents and Equipment Needed but not Supplied

- Tributylphosphine (Product Code T7567) and Iodoacetamide (Product Code A3221) or the ProteoPrep Reduction and Alkylation Kit (Product Code PROTRA)
- High purity water (Product Code W4502)
- 30 °C water bath
- Micropipettors
- Graduated cylinder
- Microcentrifuge (to 12,000 rpm)
- Laemmli 2x Sample Buffer (Product Code S3401)

Preparation Instructions

ProteoPrep Blue Albumin and IgG Depletion Medium is supplied as a slurry in 25% (v/v) ethanol with 75 mM NaCl containing 60% packed medium. The medium in the bottle must be completely resuspended prior to removing an aliquot.

Note: The following solutions will become cold to the touch and need to be warmed to 20–25 °C to dissolve completely. A 30 °C water bath will aid in the dissolution of the material. **Do not allow the temperature of the solution to rise above 30 °C, since these products may begin to form cyanates that will be detrimental to the proteins.** Aliquot the unused solution into 1–2 ml volumes and freeze at −20 °C for up to 6 months.

Add 40 ml of high purity water to the contents of the ProteoPrep Blue Equilibration Buffer container. The final volume is 50 ml.

Add 15 ml of high purity water to the contents of the Protein Extraction Reagent Type 4 container. The final volume is 23 ml.

Storage/Stability

The components in this kit will remain stable for at least 1 year in their unopened containers.

Procedure

A 0.4 ml aliquot of the medium slurry from the ProteoPrep Blue Albumin and IgG Depletion Kit will remove greater than 85% of the albumin and 70% of the IgG from a 75 µl serum sample as determined by ELISA. A greater depletion of albumin and IgG can be expected with a smaller serum sample (25–50 µl), due to a lower sample to medium ratio. Because of the high affinity of the medium for albumin and IgG, less binding of the proteins of interest (non-albumin and non-IgG) can be expected with a larger (50–100 µl) serum sample as the binding sites become saturated with albumin.

This kit is designed for using a serum volume of 25–100 µl per 0.4 ml of the ProteoPrep Blue Albumin and IgG Depletion Medium. Sufficient medium is provided for the analysis of 25 samples. If the serum volume is less than 25 µl, it is recommended to use only 0.2 ml of suspended medium slurry. However, only 25 spin columns are provided with the kit. Additional spin columns (Product Code SC1000) may be purchased.

Column Equilibration

1. Gently resuspend the medium by swirling until no settled medium remains on the bottom of the bottle when inverted and a uniform suspension is formed.
2. Transfer a 0.4 ml aliquot of the suspended medium slurry to a spin column.
   Note: For each spin column ensure that you swirl the medium immediately before removing an aliquot to prevent settling of the medium.
3. Centrifuge the spin column in the 2 ml collection tube at 10,000 rpm (8,000 x *g*) for 5–10 seconds to remove the storage solution.
4. Add 0.4 ml of Equilibration Buffer to the medium in the spin column and centrifuge at 10,000 rpm (8,000 x *g*) for 5–10 seconds. Discard the buffer in the collection tube and place the spin column back into the same collection tube.
5. Add another 0.4 ml of Equilibration Buffer to the medium in the spin column and centrifuge at 10,000 rpm (8,000 x *g*) for 20–40 seconds. Discard the buffer in the collection tube and place the spin column into a fresh collection tube.

Serum Depletion of Albumin and IgG

1. Add the serum sample (25–100 µl) to the top of the packed medium bed and incubate at room temperature for 5–10 minutes. The sample will immediately adsorb into the medium ensuring efficient binding and minimal sample dilution.
2. Centrifuge the spin column and collection tube at 10,000–12,000 rpm (8,000–12,000 x *g*) for 60 seconds.
3. Reapply the eluate in the collection tube to the top of the medium bed. Incubate for 5–10 minutes. This step removes an additional 5% of albumin.
4. Centrifuge the spin column in the same collection tube as before for 60 seconds.
5. The “two times depleted” serum should remain in the collection tube and should be pooled with the first wash step (step 6) for optimal protein recovery.
6. Wash the remaining unbound proteins from the spin column by adding 100 µl of Equilibration Buffer to the top of the medium bed, centrifuge for 60 seconds, and pool with the flow through from step 4. The majority (>95%) of the unbound proteins will be in this pool of depleted serum.
7. Optional - It is recommended that a second wash of the column be performed and analyzed by SDS-PAGE. Typically, a trace amount of albumin and IgG may be seen in a second wash. It is not recommended that the second wash be pooled with the depleted serum from step 6, unless a significant amount of unbound protein is seen. Pooling the second wash with the depleted serum from steps 3–6 may lead to unnecessary dilution of the depleted serum. Place the spin column in a fresh collection tube. Add 0.15 ml of Equilibration Buffer to the top of the packed medium and centrifuge at 10,000–12,000 rpm (8,000–12,000 x *g*) for 60 seconds.
8. The albumin/IgG depleted serum from steps 3–6 may be stored at −20 °C for long-term storage.

Elution of Bound Proteins (optional)

A small number of proteins, in addition to albumin and IgG, may bind to the medium. It is recommended that these bound proteins also be analyzed the first time that the ProteoPrep Blue Albumin and IgG Depletion Kit is used to ensure that the protein(s) of interest are not bound. The bound proteins extracted with the Protein Extraction Reagent Type 4 is 2D electrophoresis compatible. For SDS-PAGE, carry out the following steps using a 2X Laemmli Sample Buffer (Product Code S 3401), which may be purchased separately.

1. Transfer the column to a new collection tube.
2. Add 150 µl of Protein Extraction Reagent Type 4 to the top of the packed medium and centrifuge for 60 seconds.
3. Add a second 150 µl aliquot of Protein Extraction Reagent Type 4 to the top of the packed medium and centrifuge for 60 seconds. This is pooled with the 150 µl sample from the previous step. The pooled 300 µl of extract contains at least 95% of the bound proteins (albumin and IgG).
4. It is recommended to dilute an aliquot of the bound protein extract immediately for either SDS-PAGE or 2D electrophoresis and then store at −20 °C. The remainder of the bound protein extract should be stored at 2–8 °C, because freezing of the concentrated bound protein extract may cause the proteins (albumin) to precipitate.

Two-Dimensional Electrophoresis Sample Preparation

1. The appropriate sample volume for 2D electrophoresis will be determined by the gel staining procedure to be used (Coomassie® Brilliant Blue or silver staining). For a Coomassie stained gel, a proportion of the depleted serum corresponding to 5–20 µl of original serum is recommended. For a silver stained gel, a proportion of the depleted serum corresponding to 0.5–2 µl of original serum is recommended.
2. Dilute an aliquot of the depleted serum or bound protein fraction with the Protein Extraction Reagent Type 4 to the volume recommended for IPG strip rehydration by the strip manufacturer, typically 125 µl for 7 cm strips, 200 µl for 11 cm strips, and 300 µl for 18 cm strips. **The volume of depleted serum should be ≤25% of the final dilution volume to ensure sufficient protein solubilization for focusing.**
3. It is recommended that the sample be reduced and alkylated prior to the first dimension (IPG strip). This step can be performed using the ProteoPrep Reduction and Alkylation Kit (Product Code PROTRA).
   Alternatively, the following method may be used: For reduction with 5 mM tributyl phosphine using Product Code T7567 (0.2 M), add 1 µl for every 40 µl of diluted sample and incubate at room temperature for 30–60 minutes. Following reduction, alkylate with 15 mM iodoacetamide using Product Code A3221 (0.5 M) by adding 1 µl for every 30 µl 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. The majority of the proteins will focus within the pH 4-7 range.

Troubleshooting Guide

Problem	Possible Cause	Solution
Poor albumin and/or IgG extraction	Large serum load	Decrease the serum volume.
	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 serum volume.
	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).

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