ITSI BIOSCIENCES

Depletion of Albumin (Alb) from Serum or Plasma Samples Using the *ITSIPREP* ™ Albumin Segregation Kit Column (ASKc)*

IMPORTANT: K-0012-10 is a validated kit and procedure for depletion of albumin (Alb) from serum and plasma samples and analysis of the albumin enriched fraction (Alb+) and/or albumin depleted (Alb-) fractions by electrophoresis or mass spectrometry. More than 90% of albumin is depleted from whole serum using the *ITSIPrep* ASKc Kit (Figure 1). This procedure has been tested and successfully applied in our laboratory and found to improve the chances of revealing less abundant proteins in the Alb- fraction or albumin-associated proteins in the Alb+ fraction. Exercise extreme caution when working with proteins and protect your protein sample from breakdown and contamination by wearing gloves and placing tubes on ice. Work with clean equipment and in a clean/enclosed environment to prevent the introduction of common airborne contaminants such as keratin.

Read the procedure completely and assemble all materials needed before starting.

MATERIALS provided in the Kit:

Item	Size	Catalog #	Storage
Albumin Segregation Matrix (ASM)			
ASM Buffer 1	NA	Cat#:K-0012-10.1	Rm. T.
ASM Buffer 2	NA	Cat#:K-0012-10.2	Rm. T.
ASM Buffer 3	NA	Cat#:K-0012-10.3	Rm. T.
Microspin Columns containing ASM w/tubes	10 x 1.0mL	Cat#:K-0012-10.4	**4°C**.
5KDA MWCO Centrifugation Device	20 x 0.5mL	Cat#:K-0012-10.5	Rm. T.
Micro Centrifuge Tubes	30 x 1.5mL	Cat#:K-0012-10.6	Rm. T.
Procedure			

MATERIALS REQUIRED but not supplied:

1. Refrigerated centrifuge
2. Ice bucket and wet ice
3. Adjustable pipette (Use recently calibrated adjustable pipettes to ensure accuracy)
4. Vortex mixer

Procedure:

- Add 100mL of MilliQ grade water to **ASM Buffer 1**, **ASM Buffer 2**, and **ASM Buffer 3** to obtain the working **Buffer 1**, **Buffer 2**, & **Buffer 3**.
- Mix well to completely dissolve the salts and store the buffer at 4° C. **Discard unused buffers after 2 weeks of storage at 4°C.**

A. Albumin Segregation:

1. Place a Macrospin column in a 2.0mL micro centrifuge tube, which has the lid removed.
2. Centrifuge the Macrospin column at 2000 rpm for 5 seconds to remove the excess storage buffer. DO NOT ALLOW THE MATRIX TO DRY by centrifuging for too long. If the ASM looks too dry reduce the centrifugation time to 3 seconds for future spins or if too wet increase centrifugation time to 8 or 10 seconds. DISCARD THE FLOW THRU.
3. Add 500 µL of **Buffer 1** to the Macrospin column, and repeat step 2.
4. Repeat step 3 two more times.
5. In the provided 1.5mL micro centrifuge tube add serum or plasma sample containing no more than 5mg of total protein.
6. Bring the volume up to 600 µL with **Buffer 1** and Mix by gentle vortexing.
7. Transfer the entire mixture (~600 µL) with a pipette to the top of the washed ASM in the Macrospin column. **Be sure the macrospin column is inside a 2.0mL micro centrifuge tube.**
8. Allow the Macrospin column, containing the sample, to sit on the bench top at room temperature for 1 minute.
9. Centrifuge the Macrospin column for 5 seconds. **DO NOT ALLOW THE MATRIX TO DRY.**
10. Transfer the flow thru into the same Macrospin column with a pipette and repeat steps 8 and 9.
11. Transfer the flow thru to a clean 1.5mL micro centrifuge tube, and place on ice until next step.
12. Pipette 300 µL of **Buffer 1** to the top of the matrix in the macrospin column, and centrifuge for 5 seconds at 2000rpm. This is to wash the column.
13. Collect the flow thru and add it to the micro centrifuge tube containing the flow thru from step 12. Store at –80°C until needed or place on ice until next step. Start at step 14 for Alb- fraction and Step 19 for Alb+ fraction.

B. Processing of the Albumin Depleted (Alb-) fraction prior to 2D-DIGE:

Concentration, Desalting & Buffer Exchange:

14. Transfer ~500 µL of the albumin depleted sample to a 5KDa MWCO centrifugation device.
15. Centrifuge at 12,000g (preferably at 4°C) for 15minutes or until the sample is concentrated to ≤25 µL.
16. Add 300 µL of **Buffer 2** to the concentration device and mix by repeated pipeting of the mixture up and down. Centrifuge at 12,000g until the sample is concentrated to ≤25 µL.
17. Transfer the concentrated sample (~25 µL) to a storage tube.
18. Repeat steps 14-17 with the remaining depleted sample. If the albumin enriched fraction is being discarded, 2 centrifugation devices per sample can be used to reduce the processing time.
19. Place on ice and start the 2D-DIGE process immediately or store sample at -80° C until needed.

C. Processing of the Albumin enriched Fraction (Alb+) prior to 2D-DIGE:

20. Elute the bound proteins from the ASM by placing the column with the ASM in a clean 2.0mL micro centrifuge tube, and add 400 µL of **Buffer 3** to the beads. Incubate at room temperature for 10minutes. The buffer should flow through the matrix by gravity. A quick spin in a centrifuge may be needed if the buffer does not flow through.
21. Repeat step 19.

22. Transfer the flow through to a clean 1.5mL micro centrifuge tube and store at -80°C until needed, or proceed to step 22.

Concentration, Desalting & Buffer Exchange:

23. Transfer 500 µL of the albumin enriched sample to a 5KDa MWCO centrifugation device.
24. Centrifuge at 12,000xg to concentrated sample to ~50 µL. **Note**: The concentration should not take more then 30 minutes and it may not be possible to concentrate the sample below 50 µL.
25. Add 400 µL of **Buffer 2** to the concentrated sample in the concentration device, and mix by repeated pipeting of the mixture up and down.
26. Centrifuge the sample as in step 23.
27. Repeat steps 24 and 25 two more times.
28. Transfer the concentrated sample (~50 µL) to a storage tube.
29. Repeat steps 23-28 with the remaining albumin enriched sample.
30. Place on ice and use immediately or store sample at -80° C until needed.

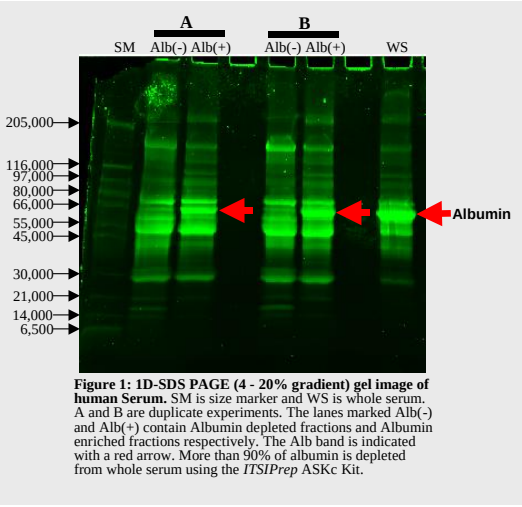

Figure 1: 1D-SDS PAGE (4 - 20% gradient) gel image of human Serum. SM is size marker and WS is whole serum. A and B are duplicate experiments. The lanes marked Alb(-) and Alb(+) contain Albumin depleted fractions and Albumin enriched fractions respectively. The Alb band is indicated with a red arrow. More than 90% of albumin is depleted from whole serum using the *ITSIPrep* ASKc Kit.

***Conditions for use of this protocol/Buffers:**

This VBP is the intellectual property of ITSI Biosciences. Only complete set of reagents provided by ITSI Biosciences should be used when possible because their compatibility with the downstream application has been validated. Considering that many factors can cause experiments to fail, ITSI Biosciences cannot guarantee that the use of this VBP and buffers will lead to a successful experiment. In no event shall ITSI Biosciences be held liable for loss of samples, failure of experiments or any other damage or injury associated with the use of this procedure or associated materials and reagents.

General Safety Information: Consider all chemicals as potentially hazardous. Only trained laboratory personnel familiar with good laboratory practice should handle this product. Protective clothing should be worn. Use caution to avoid contact with skin and eyes. If contact should occur, wash immediately with water and follow established guidelines/procedures in your laboratory. **Warning: Intended for research use only, not for use in human, therapeutic or diagnostic applications. The end user is responsible for all local, state and federal regulations associated with the use and disposal of laboratory reagents.**

ASM is suspended in 0.02% Sodium Azide (NaN_3). No special measures are required (other than those recommended for handling of standard laboratory chemicals) for handling or storage and no special precautions are necessary if handled correctly. The product does not appear to be toxic or have any harmful effect according to the information provided by the manufacturer. However disposal should be handled by hazardous waste disposers and according to official regulations.

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