ITSI BIOSCIENCES

Depletion of Albumin (Alb) from Serum or Plasma Samples Using the *ITSIPREP*™ Albumin Segregation Kit – Solvent (ASKs)

IMPORTANT: K-0013-50-INT is a validated and cost effective kit and procedure for partial depletion of albumin (Alb) from serum and plasma samples and analysis of the albumin enriched fraction (Alb+) and albumin depleted (Alb-) fractions using a solvent based procedure. More than 85% of albumin is depleted from whole plasma using the *ITSIPrep* ASKs Kit and Protocol. This procedure has been tested and successfully used to improve the chances of revealing lesser 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
Buffer 1	1 x 5g	Cat#: K-0013-50.1	Rm. T.
Buffer 2 bottle for acetone	1	Cat#: K-0013-50.2	Rm. T.
Buffer 3	2 x 10mL	Cat#: K-0013-50.3	-20°C
ProPreCip	1 x 0.5mL	Cat#: K-0013-50.4	4°C
Micro Centrifuge Tubes	100 x 1.5mL	Cat#: K-0013-50.5	Rm. T.
Procedure			

MATERIALS REQUIRED BUT NOT SUPPLIED:

1. Acetone-reagent grade.
2. Refrigerated centrifuge
3. Ice bucket and ice
4. Adjustable pipette (Use recently calibrated adjustable pipettes to ensure accuracy)
5. Vortex
6. Freezer
7. Disposable pestles with microtubes (optional)

PROCEDURE:

- Add 50ml of Acetone to the bottle labeled **Buffer 1**.
- Add 50ml of Acetone into the provided bottle labeled **Buffer 2**.
- Add 90ml of Acetone into each bottle labeled **Buffer 3**. Buffer 3 can now be stored at room temperature or -20°C.
- Chill **Buffer 1** on ice for at least 30 minutes before beginning.
- Chill **Buffer 2** and **Buffer 3** at -20° C for at least 30 minutes before beginning.

1. After thawing serum or plasma, centrifuge the sample in an appropriate tube at high speed (~10,000xg) for 5 minutes to precipitate any particulates.
2. Transfer a volume of the serum or plasma sample to the supplied micro centrifuge tubes, and place on ice. Any volume can be used, but a good amount if available is 50 µL.
3. Add the ice cold **Buffer 1** at 4x the volume of sample used. For example, if 50 µL is used, 200 µL of Buffer 1 should be added.
4. Vortex the tube and incubate at –20°C for 30 minutes.
5. Centrifuge the sample at 4° C for 5 minutes at 15,000xg.
6. Carefully remove the supernatant and transfer it to a micro centrifuge tube, and place on ice. **This is the fraction that is enriched with albumin (Alb+). To analyze this fraction proceed to step 15.**
7. Briefly spin the tube containing the pellet [**This is the albumin-depleted (Alb-) fraction**], and carefully remove any remaining supernatant with a pipette, and discard.
8. Add 1mL of chilled **Buffer 3** to the pellet followed by 5 µL of **ProPreCip**.
9. Vortex vigorously and incubate at -20°C for 15 minutes. Vortex at least 2x during this incubation.
10. Centrifuge as in step 5.
11. Remove and discard the supernatant.
12. Add 1mL of **Buffer 3** and repeat step 9.
13. Centrifuge as in step 5.
14. Discard the supernatant and air-dry the pellet to evaporate excess wash reagent. DO NOT OVER DRY. Re-suspend the albumin-depleted pellet in an appropriate solubolization buffer, and store at -80°C if not being used immediately.

Albumin Enriched Fraction:

15. Add 1mL of chilled **Buffer 2** followed by 5uL of **ProPreCip**.
16. Vortex and incubate at -20°C for 30 minutes. Vortex at least 2x during the incubation.
17. Centrifuge the tube at 4° C for 5 minutes at 15,000xg.
18. Carefully remove and discard the supernatant and add 1mL of the **Buffer 3** to the pellet.
19. Vortex vigorously and incubate at -20°C for 15 minutes. Vortex at least twice during the incubation.
20. Centrifuge as in step 17.
21. Remove and discard the supernatant, and add 1mL of **Buffer 3**.
22. Repeat step 19.
23. Centrifuge as in step 17.
24. Remove the supernatant and air dry the pellet to remove excess wash reagent, but don't over dry. Re-suspend the albumin-enriched pellet in the appropriate solubolization buffer, and store at -80° C if the sample isn't used immediately. The pellet should easily dissolve.

STORAGE:
ITSI Biosciences recommends that cell pellets or protein lysates be stored at –80°C in screw capped tubes until needed.

***Conditions for use of this procedure/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.**

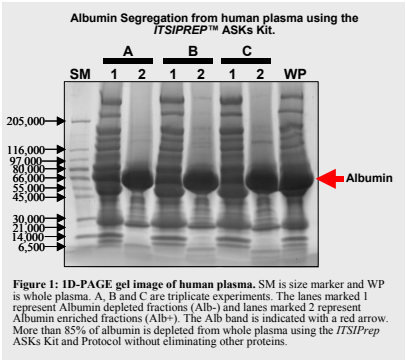

Figure 1: 1D-PAGE gel image of human plasma. SM is size marker and WP is whole plasma. A, B and C are triplicate experiments. The lanes marked 1 represent Albumin depleted fractions (Alb-) and lanes marked 2 represent Albumin enriched fractions (Alb+). The Alb band is indicated with a red arrow. More than 85% of albumin is depleted from whole plasma using the *ITSIPrep* ASKs Kit and Protocol without eliminating other proteins.

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