ITSI BIOSCIENCES

URINE PROTEIN ISOLATION AND CONCENTRATION USING THE *ITSIPREP™* TOTAL PROTEIN ISOLATION-URINE KIT (ToPI-U)*

IMPORTANT: K-0019-10-INT is a validated kit specifically developed for isolation and concentration of proteins from urine prior to downstream analysis e.g. by electrophoresis and western analysis. The ToPI-U kit contains optimized and ready-to-use reagents and concentration devices for processing of up to 10 urine samples. Up to 200 µg of total protein can be recovered from 1ml of urine. 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 K-0019-10 KIT:

Item	Size	Catalog No.	Storage
Solution 1	1 x 1.0g	Cat#: K-0019-10.1	Rm. T.
Solution 2	1 x 1mL	Cat#: K-0019-10.2	Rm. T.
Solution 3	1 x 1mL	Cat#: K-0019-10.3	Rm. T.
3000 Da MWCO and Collection Tubes	10 x 0.5mL	Cat#: K-0019-10.4	Rm. T.
Micro Grinder Pestle and Tubes	10 x 1.5mL	Cat#: K-0019-10.5	Rm. T.

#This is not your working solution. See instructions below on how to prepare the working solution.

MATERIALS REQUIRED but Not supplied:

1. Ice bucket with ice.
2. Centrifuge.
3. Vortex.
4. Micro Centrifuge Tubes.
5. Reagent grade Acetone.

NOTE:

- **A precipitate may form when urine is chilled.**
- **The cellular and total protein concentration of urine will vary based on a number of factors, including the experimental model and physiological status.**
- **Total protein recovered will vary depending on the starting volume, experimental model and physiological status of the subject.**
- **The kit is optimized for isolation and concentration of proteins that are ≥ 3000 Da.**

PREPARATION OF WORKING SOLUTIONS:

- Add 8ml of Acetone to the bottle labeled Solution 1. Mix properly to dissolve. Transfer the entire content into a 10ml volumetric flask (or measuring cylinder) and add more Acetone to bring the volume to 10ml. This should be used at all steps that require Solution 1.
- Add 9ml of Acetone to the bottle labeled Solution 2 and 9ml also to the bottle labeled Solution 3.
- Place Solution 1, 2, and 3 on ice before starting, and during use.

PROCEDURE:

1. Use an appropriately sized tube to clarify 0.1ml - 6mL of each urine sample by centrifugation at 6000rpm - 12,000rpm for 10 minutes. If centrifuge size is limiting, use four (4) 1.5mL centrifuge tubes to clarify the sample in batches. **The higher the centrifugation speed, the lesser the amount of cellular debris that will be present in the supernatant**.
2. Transfer the supernatant from Step 1 to a fresh tube. Pool samples if multiple tubes were used for clarification.
3. Use the 3000 Da concentration device provided to concentrate up to 2mL of clarified sample. Centrifuge at 12,000rpm, at room temperature for 30 min or more. The final volume should be 200ul or less from a 2ml urine sample. **Note: The concentration device holds only 500 µL. Thus, you will need to use the same device 4 times to concentrate 2ml of sample.**
4. Transfer a standard aliquot (e.g. 200 µL) of the retained **clarified and concentrated urine sample (CCUS)** to a centrifuge tube. **Use the tube supplied with the pestle at this stage. DO NOT DISCARD THE FLOW-THROUGH IF YOU ARE INTERESTED IN PROTEINS THAT ARE <3000 Da.**
5. Add 4x the volume of **Solution 1** to the CCUS. E.g. add 800uL of Solution 1 to 200 µL of **CCUS**.
6. Incubate tube on ice for 15 minutes.
7. Centrifuge (room temp) for 5 minutes at 12,000 rpm.
8. Remove and discard the supernatant. If necessary re-centrifuge for a few seconds to enable the complete removal of all the supernatant.
9. Add 1mL of ice cold **Solution 2**.
10. Mix properly by vortexing. **Note**: Pellet may not re-suspend or detach from the wall of the tube easily. This will not affect your result.
11. Incubate at –20°C for 30 minutes. V**ortex at least once during the 30 min incubation.**
12. Centrifuge as in Step 7.
13. Carefully remove and discard the supernatant.
14. Add 1mL of **Solution 3.**
15. Vortex and incubate for 15 minutes at –20°C. **Vortex once during the 15 min incubation.**
16. Carefully remove the supernatant without disturbing the pellet.
17. Allow pellet to air dry until solvent is not visible. **Over drying the pellet will make reconstitution more difficult.**
18. Reconstitute pellet with an appropriate 25 µl – 100 µl of a suitable buffer. The buffer used added should be suitable for the down stream application. The volume of buffer used should be varied as a function of the starting volume of urine and desired concentration of protein per µl. The more the buffer volume, the lower the concentration of protein per µ`l.
19. Use the Pestle provided to fully re-suspend the pellet before analysis or storage.

STORAGE:

Stored at –20°C in screw-capped tubes until analyzed.

***CONDITIONS FOR USE OF THIS PROCEDURE/SOLUTIONS:**

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 solutions 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.**

Distributed Exclusively By: