



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

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IMPORTANT: K-0019-10 is a validated kit and standardized procedure 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 up to 10 urine samples. 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 KIT:

Item	Size	Catalog #	Storage
Solution 1	1 x 10mL	Cat#: K-0019-10.1	Rm. T.
Solution 2	1 x 10mL	Cat#: K-0019-10.2	Rm. T.
Solution 3	1 x 10mL	Cat#: K-0019-10.3	Rm. T.
5000 KDa 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.

MATERIALS REQUIRED but Not supplied:

1. Ice bucket with ice
2. Centrifuge
3. Vortex
4. Micro Centrifuge Tubes

NOTE:

- A precipitate will usually form when urine is chilled.
- The cellular and total protein concentration of urine will vary based on a number of factors, including the health status of the donor.
- Place Solution 1, 2, and 3 on ice before starting.

PROCEDURE:

1. Use an appropriately sized tube to clarify up to 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 6mL of sample. **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.
3. Concentrate up to 2mL clarified sample (~10 fold) by high-speed centrifugation at 12,000rpm at room temperature using the 5,000 KDa MWCO concentration device provided. **Note: The concentration device holds only 500uL. Thus, you will need to use the same device 4 times for each sample. Centrifugation time will vary (increase) with each repeat.**
4. Transfer an aliquot (~200uL) of the retained **clarified and concentrated urine sample (CCUS)** to a centrifuge tube. **Use the tube supplied with the pestle at this stage.**
5. Add 4x the volume of **Solution 1** to the CCUS. E.g. add 800uL of Buffer 1 to 200uL 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 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. **Mix by vortexing 2 to 3 times during the 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 at least once during the 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 buffer. **The buffer added should be suitable for the down stream application.**
19. Use the Pestle provided to fully re-suspend the pellet before analysis or storage.

STORAGE:

ITSI recommends that protein samples be stored at -80°C in screw-capped tubes until analyzed.

*CONDITIONS FOR USE OF THIS PROCEDURE/SOLUTIONS:

This VBP is the intellectual property of ITSIBiosciences. Only complete set of reagents provided by ITSIBiosciences should be used when possible because their compatibility with the downstream application has been validated. Considering that many factors can cause experiments to fail, ITSIBiosciences cannot guarantee that the use of this VBP and solutions will lead to a successful experiment. In no event shall ITSIBiosciences 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.**

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