ITSI BIOSCIENCES
FAST, ACCURATE, EASY- TO-USE

TOTAL PROTEIN PRECIPITATION USING THE ToPREP KIT

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IMPORTANT: K-0016-200 is a validated kit and procedure for total protein precipitation using the ToPREP kit. The kit allows you to precipitate proteins from 200 preparations. Using the ITSIBIO ToPREP kit protein recovery >95% can be achieved. 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. Depending on the re-suspension buffer used, the precipitated proteins can be analyzed using many approaches including mass spectrometry, electrophoresis and western blotting.

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

MATERIALS PROVIDE IN THE KIT (Sufficient for 20 preparations):

Item	Size	Catalog #	Storage
Buffer 1	1 x 60mL	Cat #: K-0016-200.1	4°C
Buffer 2	1 x 60mL	Cat #: K-0016-200.2	4°C
Buffer 3	1 x 8.0mL	Cat #: K-0016-200.3	4°C
Buffer 4	4 x 50mL	Cat #: K-0016-200.4	-20°C
ProPreCip Procedure	1 x 1.0mL	Cat #: K-0016-200.5	4°C

MATERIALS REQUIRED but Not supplied:

1. Refrigerated Centrifuge
2. Ice Bucket
3. Adjustable Pipette (Use recently calibrated adjustable pipettes to ensure accuracy)
4. Vortex
5. Ultrapure Distilled/Deionized water
6. –20°C Freezer
7. 1.5mL MicroCentrifuge Tubes

PROCEDURE:

Before starting:

- Chill **Buffer 1** and **Buffer 2** on ice. Place on ice at all times during the process.
- Chill **Buffer 4** at -20°C for at least 30 minutes before use.

1. Place up to 100 µL of sample to be precipitated in a clean 1.5mL microcentrifuge tube. Place the tube on ice.
2. Add 300 µL of **Buffer 1** and vortex briefly to mix.
3. Incubate on ice for 10 minutes.
4. Add 300 µL of **Buffer 2** and vortex briefly to mix.
5. Incubate on ice for 15 minutes.
6. Centrifuge for 5 minutes at 15,000xg at 4°C.
7. Carefully remove all the supernatant by pipetting. **Do Not Disrupt the pellet.**
8. Re-centrifuge for 30 seconds and carefully remove any remaining supernatant.
9. Add 40 µL of **Buffer 3** and vortex vigorously. Place the tube on ice for 3-5 minutes.
10. Centrifuge at 15,000xg for 5 minutes at 4°C.
11. Carefully remove the supernatant with a pipette. Avoid disturbing the Pellet.
12. Re-centrifuge for 30 seconds and carefully remove any remaining supernatant.
13. Add about 25 µL of **ultrapure water**. If the level of water is below the top of the pellet add enough water to cover the pellet. Vortex vigorously. **Note that it may not be possible re-suspend the pellet at this stage. This will not affect the process.**
14. Add 1mL of chilled **Buffer 4**.
15. Add 5 µL of **ProPreCip**. **Note that the use ProPreCip is recommended for maximum recovery.**
16. Vortex briefly to mix and incubate for 30 minutes at -20°C (may be left overnight if that's more convenient). Vortex at least 2 times during the 30 minute incubation.
17. Centrifuge at 15,000xg for 5 minutes at 4°C. Remove the supernatant and discard.
18. Allow the pellet to air dry briefly. **It will be easier to redissolve the pellet if the pellet is not allowed to completely dry out.**
19. Re-suspend pellet in an appropriate buffer and use immediately or store as recommended below. The re-suspension buffer of choice should be appropriate for solubilization of proteins and compatible with the intended downstream application.

STORAGE:

Store protein isolate in appropriate tubes at -80°C.

***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 they are compatible with many downstream steps. 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 or its distributors be held liable for loss of samples, failure of experiments or any other damage or injury associated with the improper 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. **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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