



## TOTAL PROTEIN PRECIPITATION USING THE ToPREP KIT

©ITSIBIO2007: Validated BioLab Procedures-0016-10 - INT

**IMPORTANT:** K-0016-10 is a validated kit and procedure for total protein precipitation using the ToPREP kit. Using the ToPREP kit protein recovery >95% can be achieved routinely. 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. This kit will allow you to precipitate/concentrate proteins from up to 10 preparations.

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

**MATERIALS PROVIDE IN THE KIT** (Sufficient for 10 preparations):

ITEM	SIZE	CATALOG #	STORAGE
Buffer 1	1 x 4.0mL	K-0016-10.1	4°C
Buffer 2	1 x 4.0mL	K-0016-10.2	Rm. T.
Buffer 3	1 x 500µL	K-0016-10.3	4°C
Buffer 4	1 x 1.5mL	K-0016-10.4int	Rm. T.
ProPreCip	1 x 60µL	K-0016-10.5	4°C
Micro Grinder Pestle and Tubes	10 x 1.5mL	K-0016-10.6	Rm. T.
Procedure			

**MATERIALS REQUIRED but Not supplied:**

1. Refrigerated Centrifuge.
2. Ice.
3. Adjustable Pipette (Use recently calibrated adjustable pipettes to ensure accuracy).
4. Vortex.
5. Ultra pure distilled/deionized water.
6. Acetone (Reagent grade).
7. -20°C Freezer.

**PROCEDURE:**

Before starting:

- Chill **Buffer 1** and **Buffer 2** on ice. Place on ice at all times during the process.
- Add 13.5ml of Acetone to **Buffer 4** to obtain 15ml-working Buffer. 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 the clean 1.5mL micro grinder tube provided. **It is important you use the tube provided because the pestle included in the kit is designed for the tube (see Step 19).** 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 removing the Pellet.
12. Re-centrifuge for 30 seconds, and carefully remove any remaining supernatant.
13. Add about 25 µL of **Ultra pure 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\*** and vortex briefly.
16. Incubate for 30 minutes at -20°C (may be left overnight if desired). Vortex at least 1 time 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 10 µL – 50 µL of an appropriate buffer. **This recommended volume is ONLY a guide. The smaller the volume used for reconstitution, the more the protein concentration.** Use immediately or store as recommended below. You may use the pestle provided to help re-suspend the proteins. 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.

\*Addition of **ProPreCip** is recommended for maximum protein recovery. The product is not known to interfere with many common downstream applications. We however recommend that you test potential interference before subjecting valuable samples to the ToPREP process.

**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.**

**Distributed Exclusively By:**