



PROTEIN ISOLATION FROM BLOOD CELLS USING THE ToPI-Blood KIT *

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IMPORTANT: ToPI-Blood (K-0023) is a validated kit and procedure for isolation of proteins from blood cells. The resulting isolated proteins can be analyzed by mass spectrometry, western blotting, 2D-DIGE, SDS-PAGE and other downstream applications. Exercise extreme caution when working with proteins and protect your protein sample from breakdown and contamination by wearing gloves and placing tubes on ice. For maximum stability during storage the protein sample should be aliquoted to prevent multiple freeze/thaw cycles, and should be stored at a minimum of -80°C. Work with clean equipment and in a clean/enclosed environment to prevent the introduction of common airborne contaminants such as keratin. **Do not let samples stand at room temperature longer than it is necessary to prepare samples for analysis or storage.**

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

MATERIALS PROVIDED IN THIS KIT*:

Item	Size	Catalog #	Storage
10X Buffer 1	1 x 10mL	K-0023.1	Rm. T.
Buffer 2	6 x 1.0mL	K-0023.2	-20°C
Micro Grinder Pestle and Tubes	10 x 1.5mL	K-0023.3	Rm. T.
Procedure			

*Kit is shipped at ambient temperature. This kit is for 10 isolations.

MATERIALS REQUIRED but Not supplied by ITSI:

1. Refrigerated centrifuge.
2. Ice bucket.
3. Adjustable pipette (Use recently calibrated adjustable pipettes to ensure accuracy).
4. Vortex.

PROCEDURE:

- Thaw **Buffer 2** and vortex to completely dissolve the crystals. Then place the tube on ice.
- Add to the **10X Buffer 1** bottle 90mL of Milli-Q grade water to obtain 100mL of the working buffer. **Note:** The working buffer is used in all steps that require **Buffer 1**. Place **Buffer 1** on ice always.
- For long term storage, store **Buffer 1 and 2** at -20°C.

PROTEIN ISOLATION

1. Blood should be collected in a tube containing an anti-coagulant. **We recommend a commercially available plasma collection tube such as the ITSI Proteomics Grade Plasma Tube (A-0030P) which stabilizes the proteome by preventing ex vivo changes attributable to enzymatic activity.**
2. Mix by gentle inversion of the tube about 8 times. To increase the quality of the isolated protein the collected plasma should be processed immediately if possible.
3. Aliquot 500ul of the sample into one of the provided Micro Grinder Tubes.
4. Centrifuge at 1,000xg for 10 minutes at 4°C to pellet the cells.
5. Transfer the supernatant (plasma) to a new tube for storage as needed, or discard.
6. Add 1000ul of **Buffer 1** to the tube containing the cell pellet. Re-suspend the cells by gently inverting the tube about 5 times.

7. Centrifuge the sample at 1,000xg for 5 minutes at 4°C. Remove and discard the supernatant.
8. Repeat steps 6 and 7 three more times for a total of 4 washes.
9. Add 600ul of **Buffer 2** to the washed cell pellet. Add protease inhibitor to the buffer if needed. **ITSI Protease Inhibitor Cocktail for mammalian cells (A-0014) is recommended.**
10. Homogenize the cells in the lysis buffer by using the provided Micro Grinder Pestle. Take care to keep the sample on ice.
11. Incubate sample on ice for 30 minutes; vortexing the sample at least 4 times during the incubation.
12. Centrifuge the sample at 12,000 -15,000xg for 10min at 4°C.
13. Collect the supernatant and transfer to a clean tube. This is the isolated blood cell protein. If not used immediately it should be aliquoted to avoid freeze/thaws and stored at -80°C

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 always. 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 proper disposal of laboratory reagents.**



Buffer 2 (K-0023.2)

Contains Thiourea: Limited evidence of a carcinogenic effect; possible risk of harm to the unborn child; irritating to eyes and skin; harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment; wear suitable protective clothing and gloves; avoid release to the environment.

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