ITSI BIOSCIENCES
FAST, ACCURATE, EASY- TO-USE

ENRICHMENT OF CYTOSOL, NUCLEAR AND MEMBRANE PROTEINS FROM CELLS AND TISSUE USING THE *ITSIPREP*™ ProFEK KIT*

IMPORTANT: K-0015-20 is a low-cost and validated kit and procedure for protein fraction enrichment. It allows the isolation of proteins predominant in the cytosol, nuclear and membrane regions. This procedure has been successfully applied to cell lines and tissue to allow the uncovering of low abundant region specific proteins. Work with clean equipment and in a clean/enclosed environment to prevent the introduction of common airborne contaminants. Do not let samples stand at room temperature longer than is necessary when preparing samples for analysis or storage.

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

MATERIALS PROVIDED IN THE KIT:

ITEM	SIZE	CATALOG #	STORAGE*
Cytosol Buffer 1	1 x 20mL	Cat#: K-0015-20.1	-20°C
Wash Buffer 2	1 x 20mL	Cat#: K-0015-20.2	-20°C
Nuclear Buffer 3	1 x 20mL	Cat#: K-0015-20.3	-20°C
Total Membrane Buffer 4	1 x 20mL	Cat#: K-0015-20.4	-20°C
Micro Grinder Pestle and Tubes	20 x 1.5mL	Cat#: K-0015-20.5	Rm. T.
Procedure			

*Shipped at ambient temperature

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. Protease Inhibitor
6. Micro Centrifuge Tubes
7. Storage Tubes

PROCEDURE:

- Thaw all buffers supplied and place them on ice. Be sure they are clear and no precipitate is visible.
- Aliquot out enough buffers for the day
- Add a protease inhibitor to all the buffers. Follow the instructions provided by the protease inhibitor manufacturer to determine the amount of inhibitor to be used. The protease inhibitor should be concentrated. Do not add more then 1% of the buffer volume aliquoted.

1. Transfer isolated cells to the provided micro grinder tube. If they are re-suspended in media, be sure to wash them in PBS or some other appropriate buffer to remove any media contaminates. Pellet the cells, and place on ice. If using tissue cut the tissue into small pieces. **Use approximately 10^6-10^7 cells or 50 - 100mg of tissue.**
2. Immediately add ice cold **Cytosol Buffer 1**. Use 5x the volume of the cell pellet, or tissue.
3. Homogenize (5x for cells and 10x for tissue) with a homogenization device. The pestle and tube provided is recommended. Rinse the pestles after each use in clean deionized water and set aside.
4. Incubate on ice for 15 minutes.
5. Mix by vortexing the tube briefly and then centrifuge the sample at 3,000xg for 5 minutes at 4°C.
6. Carefully collect the supernatant into a clean micro centrifuge tube. **Retain the pellet. This is the nuclear-protein enriched pellet.**
7. Centrifuge the supernatant obtained in Step 6 at high speed (16,000xg or more) for 10 minutes at 4°C, and transfer the supernatant into a clean storage tube. **This is the cytosol-protein enriched fraction**. Dispose of the pellet tube.
8. Add 500 µL of **Wash Buffer 2** to the nuclear pellet obtained in Step 6.
9. Vortex briefly and centrifuge at 3,000xg for 5 minutes at 4°C.
10. Discard the supernatant and add **Nuclear Buffer 3**. Use ½ the volume of buffer used in Step 2.
11. Vortex briefly or use the pestle to completely disrupt the pellet. Incubate on ice for 30 minutes, and vortex every 10 minutes.
12. Centrifuge for 10 minutes at high speed (16,000xg or more) at 4°C.
13. Carefully collect the supernatant and transfer to a clean storage tube. **This is the nuclear-protein enriched fraction.**
14. Wash the pellet obtained in Step 12 with **Nuclear Buffer 3**, vortex briefly and centrifuge as in Step 12. Discard the supernatant.
15. Add **Total Membrane Buffer 4**. Use the same volume of buffer used in Step 2. Use the pestle to completely disrupt the pellet.
16. Incubate on ice for 30 minutes. Vortex every 10 minutes.
17. Centrifuge at 16,000xg or more for 10 minutes at 4°C.
18. Carefully collect the supernatant and transfer to a clean storage tube. **This is the membrane-protein enriched fraction.** The remaining pellet can be discarded or retained if desired.

Note: All the samples will contain high salt concentrations and some may be too dilute. It may therefore be necessary to perform a buffer exchange, desalting and/or concentration of your samples before downstream utilization.

STORAGE if applicable:
Store all protein samples at -80°C until analyzed.

***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 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 buffers 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.**

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