ITSI BIOSCIENCES

PROTEIN ISOLATION FROM CELL LINES FOR POLYACRYLAMIDE GEL ELECTROPHORESIS (PAGE) USING THE ToPI- PAGE KIT *

IMPORTANT: K-0011-C20 is a validated kit and procedure for isolation of proteins from cell lines for PAGE. This procedure has been extensively tested and successfully applied to human and non-human cell lines and if carefully followed improves the chances of performing a successful PAGE experiment. We recommend the use of tubes and buffers supplied by ITSI or provided by vendors certified by ITSI because poor quality reagents may negatively impact the downstream PAGE process, difference in-gel analysis and biological variation analysis. 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. **Do not let samples stand at room temperature longer than it is necessary to prepare samples for PAGE 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 100mL	K-0011-C20.1	Rm. T.
Buffer 2	1 x 40mL	K-0011-C20.2	Rm. T.
iTube-A Micro Centrifuge Tubes	20 x 1.5mL	K-0011-C20.3	Rm. T.
iTube-B Screw Cap Centrifuge Tubes	20 x 2.0mL	K-0011-C20.4	Rm. T.
Procedure			

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. Homogenization device
5. Vortex

PROCEDURE:

- Vortex **Buffer 2** to completely dissolve the crystals. Then place the tube on ice.
- Add the contents of **10X Buffer 1** to 900mL of MiliQ grade water to obtain 1000mL of the working buffer. **Note:** The working buffer is used in all steps that require **Buffer 1**.
- Place **Buffer 1** on ice.
- For long-term storage, store **Buffer 1** at –20°C.

A. ATTACHED CELLS:

1. Pour off growth media.
2. Use 5mL or more of **Buffer 1** to wash the monolayer of cells 3X. Typically, 2 confluent 75 cm^2 flasks produce enough cells from which enough proteins can be isolated.
3. Add 0.5mL of **Buffer 2** to each 75cm^2 flask (double the volume added if a bigger flask is used). Use a sterile scraper to scrape the cells off the flask.
4. Transfer the cell suspension to **iTube-A** and incubate on ice for 30 minutes. Vortex 3 to 4 times during the incubation.
5. Sonicate the suspension, or use a polytron type homogenizer to break up DNA and allow a more complete protein extraction.
6. Centrifuge at 15,000xg for 10 minutes at 4°C.
7. Transfer supernatant (~0.8mL) to **iTube-B** and store at –80°C until shipped to ITSI Biosciences or analyzed in-house.

B. SUSPENSION CELLS:

1. Carefully pour the cell suspension into one or more sterile centrifuge tubes and centrifuge at 1,000xg for 5 minutes at 4°C to precipitate cells out of the growth media.
2. Wash resulting cell pellet 3X with 5mL or more of **Buffer 1.**
3. Re-suspend cell pellet in **Buffer 2** by vortexing. Use at least 4X the volume of the cell pellet.
4. Transfer the cell suspension to **iTube-A** and incubate on ice for 30 minutes. Vortex 3 to 4 times during the incubation.
5. Sonicate the suspension, or use a polytron type homogenizer to break up DNA and to allow a more complete protein extraction.
6. Centrifuge at 15,000xg for 10 minutes at 4°C.
8. Transfer the supernatant to **iTube-B** and store 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 solutions 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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