PROTEIN ISOLATION FROM CELL LINES FOR TWO DIMENSIONAL DIFFERENCE GEL ELECTROPHORESIS (2D-DIGE) USING THE ToPI- DIGE KIT *

IMPORTANT: K-0010-C10 is a validated kit and procedure for isolation of proteins from cell lines for 2D-DIGE. 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 2D-DIGE experiment. DIGE is extremely sensitive to impurities, pH, water quality, and buffers. 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 2D-DIGE 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 2D-DIGE 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-0010-C10.1	Rm. T.
Buffer 2	1 x 100mL	K-0010-C10.2	4°C
Buffer 3	5 x 1.0mL	K-0010-C10.3	-20°C
iTube-A Micro Centrifuge Tubes	10 x 1.5mL	K-0010-C10.4	Rm. T.
iTube-B Screw Cap Centrifuge Tubes	10 x 2.0mL	K-0010-C10.5	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:

- Thaw **Buffer 3** and vortex 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**, and **Buffer 2** on ice.
- For long-term storage, store **Buffer 1 and 2** 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 cm² flasks produce enough cells from which enough proteins can be isolated and used for 2D-DIGE.
3. Wash the monolayer briefly with 5mL of **Buffer 2**. Discard supernatant.
4. Add 0.5mL of **Buffer 3** to each 75cm² flask (double the volume added if a bigger flask is used). Use a sterile scraper to scrape the cells off the flask.
5. Transfer the cell suspension to **iTube-A** and incubate on ice for 30 minutes. Vortex at least 2 times during the incubation.
6. Use a polytron type homogenizer to break up DNA to allow a more complete protein extraction. **Do not homogenize for longer than 5s, and do not allow sample to heat up.**
7. Centrifuge at 15,000xg for 10 minutes at 4°C.
8. 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. Mix by shaking and 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.** Centrifuge at 1000xg to pellet and remove supernatant completely after each wash.
3. Add 5mL or more of **Buffer 2**. Vortex briefly and pellet as in Step 2 above. Remove supernatant.
4. Add at least 4X the volume of the cell pellet of **Buffer 3**. Vortex to completely re-suspend the cell pellet.
5. Transfer the cell suspension to **iTube-A** and incubate on ice for 30 minutes. Vortex at least 2 times during the incubation.
9. Use a polytron type homogenizer to break up DNA to allow a more complete protein extraction. **Do not homogenize for longer than 5s, and do not allow sample to heat up.**
6. Centrifuge at 15,000xg for 10 minutes at 4°C.
10. 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. It is provided at no cost to ITSI Biosciences' partners who intend to send isolated protein samples to ITSI Biosciences for 2D-DIGE analyses or have obtained buffer(s) from ITSI Biosciences. Only complete set of reagents provided by ITSI Biosciences should be used when possible because their compatibility with the downstream labeling step has been validated. Considering that many factors can cause 2D-DIGE experiments to fail, ITSI Biosciences cannot guarantee that the use of this VBP and buffers will lead to a successful 2D-DIGE 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 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 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.**


Buffer 3 (K-0010-C10.3)
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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