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

Important: K-0010-T20 is a validated kit and procedure for isolation of proteins from tissue prior to 2D-DIGE. This kit contains optimized buffer systems and the procedure has been extensively tested and successfully applied to human and non-human tissue 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. 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	Sixe	Catalog #	Storage
10X Buffer 1	1 x 100mL	K-0010-T20.1	Rm. T.
Buffer 2	10 x 1.0mL	K-0010-T20.2	-20°C
iTube A Micro Centrifuge Tubes	20 x 1.5mL	K-0010-T20.3	Rm. T.
iTube B Screw Cap Centrifuge Tubes	20 x 2.0mL	K-0010-T20.4	Rm. T.
Procedure			

MATERIALS REQUIRED but Not supplied:

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

PROCEDURE:

- 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.
- Thaw **Buffer 2** and vortex to completely dissolve the crystals and place the tube on ice.
- Rinse your homogenization device with **Buffer 1** once. Remove excess Buffer with a clean wipe. Rinse homogenization device twice with ddH_2O immediately before use.

1. Use approximately 50 – 100mg of tissue.
2. If the tissue is fresh it can be rinsed briefly with 5-10mL of **Buffer 1** to remove any extracellular fluids. You may dab the tissue with a clean laboratory wipe to remove excess buffer. **If the tissue is frozen avoid thawing and proceed to the next step.**
3. As quickly as possible, transfer the tissue into **iTube-A** or a suitable tube and add **Buffer 2**. Use a minimum of 3x the volume of the tissue. **Use approximately 500uL for 100mg tissue.**
4. The tissue should be rapidly homogenized taking care not to create excessive foam and minimize heating of the sample. Sample tube can be immersed in ice during homogenization to avoid heating.
5. Incubate the homogenized sample on ice for about 30 minutes. Vortex at least 3 times during the incubation.
6. Centrifuge the sample at 15,000xg for 10 minutes at 4°C.
7. Transfer supernatant to **iTube-B** and place on ice.
8. Store at −80°C if not analyzed immediately.

STORAGE:

ITSI recommends that protein lysates be stored at −80°C in **iTube-B** or equivalent tubes 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 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 2 (K-0010-T20.2)**

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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