Minute™ Total Protein Extraction Kit for Skin Tissue

Catalog number: SA-01-SK

Description

Skin tissue is consisted of epidermis, dermis and subcutaneous fat. Because of its unique structure, skin tissue is notoriously difficult to homogenize. It is also very difficult to lyse the cells in the tissue for total protein extraction. The traditional solution-based protein extraction method such as RIPA is inefficient and the yield is very low. The profile of extracted protein is also incomplete with solution-based methods. This kit provides a highly efficient method for total protein extraction from human or animal skin tissues by a combination of mechanical extraction and chemical lysis of skin tissues. The kit features a simple and rapid single tube protocol and optimized buffers for skin tissues. The researchers have the option to choose either denaturing cell lysis buffer or native cell lysis buffer, which are specifically tailored for skin tissue. The whole procedure takes less than 10 min to complete and the protein yield is in the range of 1-5 mg/ml. The materials provided are sufficient for 50 extractions.

Applications

Proteins extracted with this kit can be used for many downstream applications such as SDS-PAGE analysis, Western blotting, IP. ELISA, enzyme activity assays and proteomic analysis. The buffers are compatible with IMAC resins for his-tagged protein purification. The salts and detergents in the extracted protein sample should be removed prior to mass spectrometry analysis

Kit components

1.	Denaturing Buffer	25 ml
2.	Native Buffer	25 ml
3.	Protein Extraction Powder	5g
4.	Plastic Rod	2
5.	Filter Cartridge	50
6.	Collection Tube	50

Shipping and storage: This kit is shipped at ambient and stored at room temperature

Additional Materials Required

Table-Top Microcentrifuge with a maximum speed of 14,000-16,000 X g

Important Product Information

Denaturing buffer contains ionic detergent and other chemicals for solubilization of extracted proteins. It may form precipitate at low temperature. It is not recommended to pre-chill it on ice. Native buffer can

be pre-chilled and will not form precipitate. The lysis buffers do not contain protease inhibitors. If proteolysis is a concern, it is recommended to add protease inhibitor cocktails to aliquot of the buffers prior to use. For determination of protein concentration, BCA kit (Pierce) is recommended. To study protein phosphorylation, **phosphatase inhibitors** (such as PhosStop from Roche) must be added to the buffer prior to use.

Protocol

For demonstration purposes, following amount of starting material and lysis buffer is recommended. The protocol can be scaled up/down proportionately. Skin tissue preparation: for animal skin with fur/hairs the first step is to remove fur/hairs using a trimmer or a sharp razer. Try to dissect and remove subcutaneous fat as much as possible. Perform the protocol at room temperature.

1. Weight out 30-40 mg skin tissue (fresh/frozen) and cut it with a pair of sharp scissors into smaller pieces (1 X 1 mm or smaller). Place cut tissue in a filter cartridge with collection tube.

2. Add 50 to 80 mg protein extraction powder on top of the tissue followed by addition of 100 µl lysis buffer.

3. Immediately grind the tissue with the plastic rod provided against the surface of the filter with moderate twisting force for 2-3 min. Add another 100 µl lysis buffer to the filter and continue to grind for about 30 seconds to 1 min. **The plastic rods are reusable after cleaning.**

4. Centrifuge the tube at top speed in a microcentrifuge for 1 min. Remove and discard the filter. Transfer the supernatant to a fresh tube (this is extracted total protein). Usually a thin layer of lipid is present on top of the supernatant. Try to avoid the lipid layer by inserting the pipette tip under the lipid layer for transferring the supernatant. The white-grey pellet in the bottom of collection tube is passing through protein extraction powder that should be discarded.

Application tips: If the final protein yield is low, incubate grinded tissue in step 3 at room temperature for 5-10 min. During incubation period, the lysis buffer may drip into collection tube. This is normal and will not affect the quality of extracted protein.