



Minute™ Total Protein Extraction Kit for Bone Tissue

Catalog number: SA-02-BT

Description

Bone tissues are commonly used for research. There are two types of bone tissues: compact and spongy. Cells in bone tissues are tightly packed. Because of their unique structures it is very difficult to efficiently extract total protein from them. The traditional solution-based protein extraction method such as RIPA is inefficient and the protein yield is very low. The profile of extracted protein is also incomplete with solution-based methods. This kit provides a highly efficient method for extracting proteins from human or animal bone tissues by a combination of mechanical extraction and chemical lysis of cells in tissues. The kit features a simple and rapid single tube protocol and optimized buffers for bone tissues. The researchers have the option to choose either denaturing cell lysis buffer or native cell lysis buffer, which are specifically tailored for bone tissue. The whole procedure takes less than 10 min to complete and the protein yield is in the range of 0.5-2 mg/ml depending upon type of bones. 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, the following amount of starting material and lysis buffer is recommended. However, the protocol can be scaled up/down proportionately. Bone tissue preparation: Prior to protein extraction, the bone tissues need to be crushed into smaller pieces or powders by mechanical means (bone crusher, blender, or scissors etc.). Try to dissect and remove fat and muscle attached to the bone tissue as much as possible. Perform the protocol at room temperature.

1. Weight out 50-100 mg crushed bone tissue (fresh/frozen) and place it 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 μ l 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 μ l 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). 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.