Minute™ Total Protein Extraction Kit for Blood Vessels

Catalog number: SA-03-BV

Description

Blood vessels (arteries and veins) are made of specialized connective tissues. There are three layers of tissues in blood vessels: tunica, tunica media and endothelium. Due to the unique structures blood vessels are 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 protein yield is very low. The profile of extracted protein is also biased with solution-based (incomplete protein profile) methods. This kit provides a highly efficient method for total protein extraction from human or animal blood vessels by a combination of mechanical extraction and chemical lysis. The cell lysis buffers used are much stronger than RIPA buffer. The kit features a simple and rapid single tube protocol and optimized buffers for blood vessels. The researchers have the option to choose either denaturing cell lysis buffer or native lysis buffer, which are specifically tailored for blood vessels. The whole procedure takes less than 10 min to complete and the protein yield is in the range of 1-3 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

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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. Blood vessel preparation; carefully remove tissues attached to the blood vessel. Cut blood vessels open with scissors and rinse in cold PBS to remove blood inside. The protocol may be performed at room temperature.

1. Weight out 30-40 mg blood vessel (fresh/frozen) and cut it with a sharp blade or a pair of scissors into small 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). 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 or increase grinding time. During incubation period, the lysis buffer may drip into collection tube. This is normal and will not affect the quality of extracted protein.

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