Minute™ Protein Extraction Kit for Fixed and Embedded Tissues

Catalog number: FE-025

Description

Formalin-fixed and paraffin-embedded (FFPE) tissues are widely available and serve as a potential rich source for biomedical research. However protein extraction from FFPE tissue is very challenging due to formalin mediated molecular cross-linking. Traditional methods for protein extraction from FFPE tissues involve repeated deparaffinization and rehydration using organic solvents followed by protein extraction at high temperature with or without optional sonication step. These methods, though relatively effective in some cases, are tedious and time consuming (3-4 h). FE-025 provides a simple and rapid way to extract protein from FFPE tissue without organic solvent deparaffinization. The whole procedure can be completed in less than one hour with a protein yield of 2-3mg/ml.

Application

Extracted proteins can be used for many downstream applications such as SDS-PAGE, Western blotting ELISA, immunoprecipitation and proteomic studies.

Kit components

1. Buffer A 20 ml
2. Buffer B 20 ml
3. 1.5 ml microfuge tubes (20)
4. Pestles (2)

Shipping: This kit is shipped at ambient temperature**.**
Storage: Store the kit at RT**.**

Additional Materials Required

Table-Top Microcentrifuge with a maximum speed of 14,000-16,000 rpm
A Heat block or a water bath

Important Product information

This kit can be used for protein extraction from FFPE tissues. However the yield and resulting protein profile are fixation time and sample age dependent. Longer fixation time (>48h) and aging samples are expected to yield lower protein concentration. Protease inhibitor cocktail is recommended to be added to buffer B after the buffer is transferred to 1.5 m l microfuge tube (see step 3 below). For determination of protein concentration, detergent-compatible BCA kit (Pierce) is recommended because the extraction buffer (buffer B) contains 1% SDS. To study protein phosphorylation, phosphatase inhibitors (such as PhosStop from Roche) should be added to buffer B prior to use.

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Protein Extraction Procedure

1. Remove excessive paraffin from tissue sections. The thickness of tissue section should be10-20 μm. The size should be about 30-60 mm^2. Place 4-5 tissue sections in a 1.5-2.0 ml microfuge tube **from your Lab** and add 1 ml buffer A to the tube.
2. Heat the tube at 90-95^oC in a heat block or a water bath for 8-10 min. Inverting the tube 2-3 time and quickly remove the buffer from the tube completely when the buffer is still hot leaving the tissues in the tube.. Scrape the tissues into a fresh **1.5 ml microfuge tube provided in the kit** using a pipette tip (Note: don't use the microfuge tube from other source in this step, it may not fit well with the pestle provided).
3. Inverting buffer B bottle 20 to 30 times to mix tissue dissociation powder well and quickly transfer 200 μl buffer B to the tube containing the tissue sample (optional: it's much easier to transfer buffer B if the pipette tip is cut 2-3 mm from the tip to make the opening larger). The amount of buffer B added depends upon the number of sections used. Generally use 50 μl buffer B for each tissue section.
4. Homogenize the tissue sample with a pestle provided by grinding with twisting force for about two min. (The pestle is reusable, wash it with distilled water and dry with a piece of paper towel). Cap the tube and heat at 90-95^oC for 40 min to 1 hour in a heat block or a water bath. After heating centrifuge the tube at 14,000 rpm to 16,000 rpm for 10 min. Transfer extracted protein in the supernatant to a fresh tube for downstream application or store at -80^oC for later use.

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