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 1-2 mg/ml.

Application

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

Kit components(20T):

1. Buffer A 30 ml
2. Buffer B 10 ml
3. Protein Extraction Powder (2 g)
4. 1.5 ml microfuge tubes (20)
5. Pestles for 1.5 ml tube (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. A Heat block or a water bath

Important Product Information

This kit can be used for protein extraction from FFPE embedded tissues or non-embedded but formalin fixed tissues. Protease inhibitor cocktail is recommended to be added to aliquot of buffer B 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 buffer B prior to use.

Protein Extraction Procedure

1. Prepare thin tissue sections by a sharp blade. Remove excessive paraffin from tissue sections. The thickness of tissue section should be about 10-20 µm. The size should be about 30-60 mm^2. Place 4-5 tissue sections in a 1.5 ml microfuge tube **provided.**
2. Add 0.7 ml buffer A to the tube and heat at 95°C in a heat block or a water bath for 10 min. Quickly centrifuge at 10,000 X g for 5-10 second at RT while the tube is still hot. Quickly remove all supernatant by pipetting with a 1 ml pipette tip. Add 0.7 ml buffer A to the same tube and repeat heating and centrifugation one more time, Remove supernatant completely.
3. Add 200 ul buffer B to the tissue pellet followed by addition of 80 mg protein extraction powder to the bottom of the tube.
4. Homogenize the tissue sample with a pestle provided by grinding with twisting force for about 2-3 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°C for 40 min to 1 hour in a heat block or a water bath. After heating, centrifuge the tube at 12,000 X g for 10 min. Transfer the supernatant (extracted protein) to a fresh microfuge tube and determine the protein concentration by BCA assay. The extracted protein is ready for downstream applications. It can also be stored at -80°C for later use.

   Note: The amount of buffer B used in the protocol is recommended. It can be scaled up or down according to amount of tissue used. If protein concentration is low increase amount of starting tissue.

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