Minute™ Detergent-Free Nuclear Matrix Protein Extraction Kit

(For mammalian cultured cells/tissues)

Cat. No. NM-033

Description

Nucleoplasm, also referred to as karyoplasm/nucleus sap, is consisted of chromosomes, nucleolus and nuclear matrix. Nucleoplasm plays a central role in genetic information flow and regulation of gene expression. Nuclear protein isolation has been traditionally accomplished by extraction protocol using detergent-containing buffers, which is effective in extracting total nuclear proteins but unable to separate membrane-bound proteins and water-soluble matrix proteins. The detergent-free nuclear matrix protein extraction kit is designed to separate cellular protein into three fractions: **cytosolic, nuclear matrix and water-insoluble fraction** (mainly envelope and nucleic acid-associated proteins) using a patented spin column-based technology. The buffers used are detergent and EDTA-free. The whole protocol can be completed in less than 1h. The proteins extracted are in their native state and can be used in many downstream applications such as gel retardation, transcription factor analysis, apoptosis, and protein trafficking studies.

Kit components (20T):

1. 30 ml buffer A
2. 5 ml buffer B
3. 20 protein extraction filter cartridges
4. 20 collection tubes
5. Plastic rod (2)

Shipping: This kit is shipped at ambient temperature**.**
Storage: Store the kit at 4^{o}C upon arrival**.**

Additional Materials Required

Table-Top Microcentrifuge with a maximum speed of 14,000-16,000 X g. **The centrifuge should be able to reach maximum speed within 10 seconds.**

Important information

This kit, in general, can be used for separation of cellular proteins into three fractions. However, the purity is cell/tissue type dependent. It is recommended that proteinase inhibitors and/or phosphatase inhibitors (if protein phosphorylation is involved) are added to aliquot of buffer A and buffer B prior to the extraction. It is recommended to use BCA Protein Assay Kit for determination of protein concentration (Pierce, Cat #:23227). **Pre-chill buffers on ice prior to use and centrifugation should be performed at** 4^{o}C.

For Cultured Cells (Fresh or frozen):

1. Collect 10-30 million cultured cells by low speed centrifugation (500 X g for 3 min). Wash the cell pellet once with 1 ml cold PBS. Remove the supernatant **completely**.
2. Resuspend the pellet in 500 µl cold buffer A and incubate on ice for 5-10 min. After incubation, vortex the tube vigorously for 20-30 seconds. Transfer the cell suspension to a filter cartridge with collection tube.
3. Centrifuge in a table top microfuge at 14,000 to 16,000 X g for 30 seconds, resuspend the pellet by pipetting up and down a few times and re-pass the suspension through the same filter one more time.
4. Discard the filter and resuspend the pellet by vortexing vigorously for 10 seconds, centrifuge at 500 X g for 2 min. Remove the supernatant and save it if desired. The supernatant can be centrifuged at 16,000 X g for 30 min at 4°C. The supernatant is cytosolic fraction.
5. Wash the pellet from step 4 with 0.5 ml cold buffer A **twice** (500 x g 2 min), remove the supernatant completely. Add 50-200 µl cold buffer B to the tube and pipette up and down for 10-20 times to resuspend. Vortex vigorously for 15-20 seconds. Place the tube on ice for 10 min and vortex one more time as described. The amount of buffer B used depends on the pellet size. For cultured cells use 50-100 µl, for tissue samples use 100-200 µl. If final matrix protein yield is low, the resuspended pellet can be frozen at -80°C for 10 min. Thaw out the tube at room temperature and extract matrix protein as described above. It must be noted that freezing at -80°C can increase yield but it may also increase the cross contamination of isolated fractions.
6. Centrifuge the extract at 16,000 X g for 10-15 min and transfer the supernatant to a fresh tube (this is nuclear matrix fraction), the pellet contains water-insoluble fraction (WIF) containing mainly nuclear envelope and nucleic acid associated proteins. If extracted nucleoplasm proteins are not used right away, add protease inhibitor to the extract and store at -80°C for future use. If contamination of water-insoluble fraction by matrix protein is an issue, wash the pellet with 0.5 ml cold PBS once (16,000 X g for 5 min). The yield is typically 50-200 µg/sample depending upon cell/tissue types used.

   Note: The matrix protein extracted is in a solution with salt concentration higher than physiological condition. It may need to be diluted 1:1 with H_2O for some downstream experiments.

For Tissues (Fresh or frozen):

1. Add 20-30 mg tissue to the filter cartridge. Thaw out frozen tissue completely on ice. Add 200 µl cold buffer A to the filter, grind the tissue for about 1-2 min using the plastic rod provided (the plastic rod is reusable, clean by washing with water and dry with paper towel).
2. Add 300 µl cold buffer A to the same filter, incubate on ice for 5 min with cap open. Cap the tube and resuspend the tissue homogenate by inverting a few times. Centrifuge in a table top microfuge at 16,000 X g for 30 seconds, resuspend the pellet by vortexing and re-pass the cells through the same filter one more time. Follow step 4 to step 6 above.

Solubilization of water insoluble fraction:

The water-insoluble fraction can be resuspended in any buffer suitable for downstream applications. However, following protein solubilization reagents are recommended:

Product Name	Cat. No.	Applications
Minute™ Denaturing Protein Solubilization Reagent	WA-009	SDS-PAGE electrophoresis and Western blotting, trypsin digestion, purification of proteins with biotin labeling or histidine labeling, etc.
Minute™ Non-Denatured Protein Solubilization Reagent	WA-010	ELISA, immunoprecipitation/Co-IP, enzymatic activity determination and other applications.
Minute™ Protein Solubilization Reagent for MS	WA-011	Trypsin digestion and subsequent mass spectrometry analysis.