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Minute™ Nuclear Envelope Protein Extraction Kit

Catalog number: NE-013

Description

The nuclear envelope is a very complex membrane-protein system that is notoriously difficult to isolate and purify due to its connection to nuclear and cytoplasmic components. Traditional method of nuclear envelope isolation and purification requires a large amount of starting material and a lengthy and tedious procedure. Invent Biotechnologies Minute™ nuclear envelope protein extraction kit is the first commercial kit designed to rapidly isolate nuclear envelope and its associated proteins in native form without using density-gradient and ultra centrifugation. Due to the use of protein extraction filter cartridges, the nuclear envelope protein isolation is simple, easy and user friendly. The nuclear envelope proteins are significantly enriched in the final prep. Unlike traditional method that requires large amount of starting cells/tissues this kit starts with only 10-20 million cells and the buffers are detergent and EDTA free. The procedure can be completed in less than 45 min with a final yield of 10-50 µg protein/sample.

Applications

The kit is designed to rapidly isolate native nuclear envelope proteins from cultured cells or cells isolated from tissues for applications such as SDS-PAGE, immunoblotting, ELISA, IP, protein translocation analysis, enzyme activity assays and other applications. This kit provides the most rapid method currently available for enrichment of native nuclear envelope proteins.

Buffer Formulations: Proprietary

Kit components (50 preps)

1. 25 ml buffer A
2. 15 ml buffer B
3. 50 protein extraction filter cartridges
4. 50 collection tubes with cap

Storage: Store Buffer A and Buffer B at -20°C upon arrival.

Additional Materials Required

1 X PBS
Vortexer
Table-Top Microcentrifuge

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Important Information:

1. Read the entire procedures carefully. Thaw buffer A and buffer B completely, invert the bottles a few times and place them on ice. Chill protein extraction filter cartridge with collection tube on ice prior to use.
2. All centrifugation steps should be performed at 4°C in a cold room or in a refrigerated mirocentrifuge.
3. To study protein phosphorylation, **phosphatase inhibitors** (such as PhosStop from Roche) should be added to buffer A prior to use. The use of protease inhibitor cocktails is optional.
4. It is recommended to use BCA Protein Assay Kit for determination of protein concentration (Pierce, Cat #:23227).

Nuclear Envelope Protein Isolation Procedures

1. Place the filter cartridges in collection tubes, and incubate on ice.

2. Collect 10-20 million cultured cells or cells isolated from tissues by low speed centrifugation (500-600 X g, 5 min). Minute™ single cell isolation kit (Cat# SC-012, Invent Biotechnologies, MN) is recommended for obtaining single cell suspension from animal tissues.

3. Wash cell pellet once with 1 ml cold PBS in a microcentrifuge tube. Remove supernatant completely and resuspend the pellet in 0.5 ml buffer A. Incubate the cell suspension on ice for 10 min. Vortex the tube vigorously for 10-20 seconds. Immediately transfer the cell suspension to the filter cartridge. Cap the filter cartridge and centrifuge at 14,000 X g for 30 seconds.

4. Discard the filter and remove supernatant completely. Wash the pellet (nuclei) in 1 ml cold PBS by vigorously vortexing for 10 seconds. Centrifuge at 500 X g for 2 min. Remove PBS completely.

5. Add 300 µl buffer B to the tube and resuspend the pellet by vigorously vortexing for 10 seconds. Incubate the tube on ice for 5 min, vortex vigorously for 10 seconds. Repeat 5 min incubation and vortexing step one more time. Since different cell type shows different sensitivity to buffer B. If you encounter viscous nuclear extract, that indicates the Buffer B is too strong for your cell type. The solution to the problem is to dilute buffer B with cold 1 X PBS. The optimal dilution needs to be determined experimentally. As a starting point you can try following dilutions: 8 parts buffer B + 2 parts of PBS, 6 parts of buffer B + 4 parts of PBS, and/or 1 part of buffer B + 1 part of PBS. The general idea is to find a diluted buffer B that gives you a good nuclear envelope extraction without viscous extract.

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6. Centrifuge the tube at 5000 X g for 5 min at 4°C. Carefully transfer the supernatant to a fresh 2.0 ml microcentrifuge tube. Add 0.8 ml cold PBS to the tube and invert the tube about 10 times (this step precipitates extracted nuclear envelope).

7. Centrifuge the tube at 16,000 X g at 4°C for 15 min. Remove supernatant and save the pellet (isolated nuclear envelope). The protein yield is typically 10-50 µg/sample. If more proteins are needed simply run multiple samples and combine the final preps. The pellet can be dissolved in any detergent-containing buffers of your choice depending upon specific downstream applications. Reagents in following table are recommended for solubilization of the pellet. For isoelectric focusing (First dimension of 2D gel) we recommend to use: 7M urea/2M thio-urea/2% Chaps and 20 mM DTT (add DTT to above mix prior to use).

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.

Troubleshooting

Problem	Solution
Low protein yield	Increase starting cell numbers Increase incubation time to 15 min (step3)
Low protein activity	Keep lysate cold/add protease inhibitors
After 8,000 rpm centrifugation (step 6) the supernatant becomes too viscous to pipette	Dilute Buffer B
Contamination of nuclear envelope fraction by cytosolic proteins	Wash nuclear envelope pellet with 0.5 ml washing buffer (0.3 M NaCl in 100 mM Tris-HCl pH 9.0).