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COMPARTMENTAL PROTEIN EXTRACTION KIT

For extracting Cytoplasmic, Nuclear, Membrane and Cytoskeletal proteins from tissues and cells

CATALOG NUMBER: 2145

LOT NUMBER:

QUANTITY: 1 Kit

DESCRIPTION:

One of the challenges of functional proteomics is separation of complex protein mixtures for quantitative and differential subcellular localization analysis. This necessitates standardized and repeatable operation procedures to isolate subcellular proteomes from tissues and cells.

Millipore's Compartmental Protein Extraction Kit addresses the challenge by providing an innovative, easy-to-perform, and cost-effective method to sequentially isolate cytoplasmic, nuclear, membrane, and cytoskeleton proteins from mammalian tissues and cells based on a proprietary technique.

This kit contains enough reagents to enrich four compartmental proteins from 5 grams tissues or approximately 125 million cells. The efficiency of subcellular fractionation has been investigated by SDS-PAGE and immunoblotting of selected marker proteins.

APPLICATIONS:

- Detection of differential post-translational modifications or differential subcellular localization of target proteins
- Enrichment of low-abundance proteins for visualization and subsequent analysis
- Preparation of nuclear extract from mammalian tissues and cultured cells is crucial for studying DNA binding proteins such as transcription factors employing gel mobility shift techniques
- Preparations of cytoplasmic fractions are useful to study soluble proteins abundant in cytosol
- Preparations of membrane fractions to study membrane proteins such as receptors
- Preparations of cytoskeleton fractions to study cytoskeleton proteins

KIT COMPONENTS:

Item	Composition	Quantity (storage)
Buffer C	HEPES (pH 7.9), $MgCl_2$, KCl, EDTA, Sucrose, Glycerol, Sodium OrthoVanadate	1 x 18 mL (2-8°C)
Buffer W	HEPES (pH 7.9), $MgCl_2$, KCl, EDTA, Sucrose, Glycerol, Sodium OrthoVanadate	1 x 50 mL (2-8°C)
Buffer N	HEPES (pH 7.9), $MgCl_2$, NaCl, EDTA, Glycerol, Sodium OrthoVanadate	6 mL (2-8°C)
Buffer M	HEPES (pH 7.9), $MgCl_2$, KCl, EDTA, Sucrose, Glycerol, Sodium deoxycholate, NP-40, Sodium	6 mL (2-8°C)
Buffer CS	Pipes (pH 6.8), $MgCl_2$, NaCl, EDTA, Sucrose, SDS, Sodium OrthoVanadate	3 mL (2-8°C)
50x PI	A cocktail of protease inhibitors	0.66 mL (-20°C)

MATERIALS REQUIRED BUT NOT SUPPLIED:

- Tissue Homogenizer
- Centrifuge and Microcentrifuge
- Rotator, rocking platform for tube incubations

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PROTOCOL:

Procedures:
I. Extracting compartment proteins extraction from tissues.
II. Extracting compartment proteins from culture cells

I. Extracting compartment proteins extraction from tissues

For **tissues**, we recommend to break tissues into small pieces with a homogenizer first, then add ice cold **buffer C** and either rotate, pipette, or vortex the mixture, and keep on ice. Note: For homogenization, an automatic machine is preferred (i.e. Polytron®); however, a manual method may work fine for some soft tissues such as brain. Precool the solutions, and keep tubes and extracts on ice for best results.

For buffers C, N, M and CS, **add 50x PI to working concentration of 1x before use**. Prewarm the CS buffer to room temperature to ensure that SDS is completely redissolved

1. Weigh a small amount of desired tissue (0-1 gm), and then cut tissue block into small pieces on a prechilled plate, then pipette ice cold **buffer C** using **2.0 mL per gram tissue**. More buffer can be used if necessary. Lowering the amount used is not recommended because if the solution becomes too thick this will compromise the fractionation of the cellular components.
2. Homogenize* the tissue or cells at moderate speed (e.g. speed 4) by rotation or vortexing, for 20 seconds. Let it stand on ice for a few seconds, repeat homogenization twice more, or until solution is uniformly homogenized.
3. Rotate, or gently mix the mixture at 4°C for 20 minutes.
4. Centrifuge sample at 18,000 g at 4°C for 20 minutes. Remove and save the supernatant into another tube. The cytoplasmic proteins are in the supernatant. The pellet contains membranes, nuclei, and cytoskeletal fractions. Keep storage tubes on ice.
5. Wash and resuspend the pellet with 4 mL of ice cold **buffer W** per gram of tissue.
6. Rotate or mix at 4°C for 5 minutes.
7. Centrifuge again at 18,000 g at 4°C for 20 minutes. Carefully pour off the supernatant and discard, wipe the edge of the tube with a tissue if necessary. This wash step simply helps to reduce cross-contamination with cytoplasmic proteins and it can be repeated for increased fidelity if desired.
8. Add 1.0 mL of ice cold **buffer N** per gram of tissue to the pellet and resuspend the pellet from step 7.
9. Rotate or mix at 4°C for 20 minutes.
10. Spin at 18,000 g at 4°C for 20 minutes. Remove and save the supernatant in to another tube. The nuclear proteins** are in this supernatant. Keep storage tubes on ice.
11. Add 1.0 mL of ice cold **buffer M** per gram of tissue to the pellet and resuspend.
12. Rotate or mix at 4°C for 20 minutes.
13. Spin at 18,000 g at 4°C for 20 minutes. Remove and save the supernatant into another tube. The membrane proteins are in this supernatant. Place tube with pellet on ice.
14. Pre-warm an aliquot of **CS buffer** to room temperature or 37°C to make it clear, and

add 0.5 mL of the room temperature **CS buffer** per gram of tissue to the pellet from step 13 and resuspend the pellet.

15. Rotate or mix the solution at room temperature for 20 minutes.
16. Spin at 18,000 g at 4°C for 20 minutes. Remove and save the supernatant in to another tube on ice. Place tube with pellet on ice.
17. Immediately wash and resuspend the pellet from step 16 with 1.5 mL of ice cold **buffer C** per gram of tissue.
18. Rotate or mix at 4°C for 5 minutes.
19. Spin at 18,000 g at 4°C for 20 minutes, save the supernatant on ice.
20. Combine the supernatants from step 16 and step 19. The cytoskeletal proteins are in this mixed solution.
21. Measure the protein concentrations of the four fractions with a detergent compatible protein concentration kit.
22. Aliquot and label the proteins properly and store at -70°C. For best results avoid repeated freeze-thaws of the protein extracts.

* We recommend using IKA Ultra Turrax T25 Basic or similar model homogenizer for tissues. Manual homogenizer can also be used; the purpose of the homogenization is to get the tissue lysed completely without breaking the nuclear compartment.

** A dialysis step may be necessary if the nuclear fraction is going to be used for the gel mobility shift assay.

II. Extracting compartment proteins from culture cells

For **adherent cells**, we recommend adding **buffer C** (the extraction buffer) directly to the plates, and scrapping the cells loose with a plastic cell scraper. The mixture is then pipetted into tubes and vortexed or rocked, cooling on ice frequently. Note: Cultures should be rinsed well with PBS prior to the addition of the extraction buffer. Failure will result in high levels of FBS and protein contamination from the cell culture media, which can make western blot analysis of the lysates difficult in some cases

For **suspension cells**, we recommend adding **buffer C** (the extraction buffer) directly to the washed, prepared cell pellet, then gently pipeting or vortexing the solution. Store the cell lysate on ice and use precooled solutions for best results.

For buffer C, N, M, and CS, **add 50x PI to a working concentration of 1x before usage**.

1. Pellet the culture cells using routine cell culture techniques. Count cells and add ice cold **buffer C** at **2.0 mL per 20 million cells**. Mix well.
2. Rotate the mixture at 4˚C for 20 min.
3. Prepare a syringe with a needle gauged between 26.5 and 30. Remove the needle tip by bending the needle several times and only leave the needle base on the syringe. Pass the cell mixture through needle base 50-90 times to disrupt the cell membrane and release the nuclei from the cells. The degree of cell membrane disruption and nucleus release can be monitored under microscope. 90-95% of the nuclei should be released from the cells.
4. Spin the cells mixture at 15,000 g at 4˚C for 20 min. The cytoplasmic proteins are in

the supernatant, take out and save in another tube. Keep storage tubes on ice.

5. Resuspend the pellet with ice cold **buffer W** at **4.0 mL per 20 million cells**.
6. Rotate at 4˚C for 5 min.
7. Spin at 15,000 g at 4˚C for 20 min. Drain the supernatant.
8. Add ice cold **buffer N** at **1.0 mL per 20 million cells** to resuspend pellet from step 7.
9. Rotate at 4˚C for 20 min.
10. Spin at 15,000 g at 4˚C for 20 min. The nuclear proteins are in the supernatant, take out and save it in another tube. Keep storage tubes on ice.
11. Add ice cold **buffer M** at **1.0 mL per 20 million cells** to resuspend pellet from step 10.
12. Rotate at 4˚C for 20 min.
13. Spin at 15,000 g at 4˚C for 20 min. The membrane proteins are in the supernatant. Take out and save in another tube. Keep storage tubes on ice.
14. Pre-warm **CS buffer** at RT or 37 ˚C to make it clear. Add buffer CS at **0.5 mL per 20 million cells** to resuspend pellet from step 13.
15. Rotate at room temperature for 20 min.
16. Spin at 15,000 g at 4˚C for 20 min. The cytoskeletal proteins are in the supernatant, take out and save it in another tube. Keep storage tubes on ice.
17. Measure the protein concentrations of four fractions.
18. Aliquot and label the proteins properly, store at –70˚C.

TROUBLE SHOOTING:

1. **Cytoplasmic proteins-carryover to nuclear and membrane fractions**

   The tissue is not completely homogenized. Increase the speed and times of homogenization. For protein extraction from cells, increase the needle gauge size and increase the times of cells passing the needle base. Also repeat the washing step (step 5 in the protocol) 1-2 times to completely remove cytoplasmic proteins. Add a washing step right after nuclear protein extraction.

2. **Nuclear proteins present in the cytoplasmic fraction**

   The tissue homogenization conditions were too harsh and the cytoplasmic membrane and nuclear membrane were broken. Decrease the speed and times of homogenization. For protein extraction from cells, decrease the needle gauge size and decrease the times of cells passing the needle base.

3. **Nuclear proteins present in the membrane fraction**

   Repeat the nuclear proteins-extraction step 1-2 times before membrane proteins-extraction.

4. **Membrane proteins present in the cytoplasmic and nuclear fractions**

   The tissue homogenization conditions were too harsh and the nuclear membrane was broken.

5. **Protein degradation**

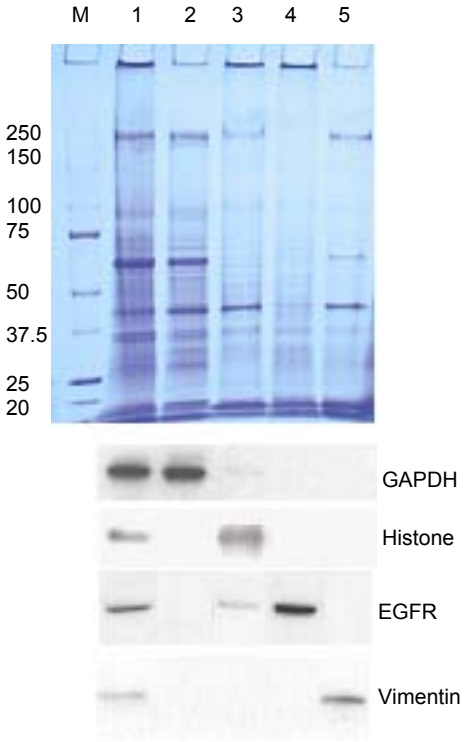
   Make sure that PI was added to Buffers C, N, M and CS before use; keep solutions on ice, work carefully, but quickly.

   a. 50x PI was not aliquoted during storage and lost activity when frozen-thawed too many

times.

b. Homogenize the tissue for less time (<20 sec); let the tube stand on ice for a few seconds before the next homogenization.

EXAMPLE OF COMPARTMENTAL PROTEIN EXTRACTION FROM MAMMALIAN TISSUE:



Total protein and compartmental proteins extracted from rat colon tissue using Millipore's Total Protein Extraction kit and the Compartmental Protein Extraction kit were subjected to SDS-PAGE in 5 identical gels. Commassie staining of one piece of gel indicated distinct protein pattern of respective fractions. Immunoblotting of PVDF membranes transferred from four other pieces of gel against cytoplasmic marker protein GAPDH, nuclear marker protein Histone H1, membrane marker protein EGFR, and cytoskeletal marker vimentin assigned the majorities of the marker proteins to their expected compartmental fractions.

Lane 1: Total protein;
Lane 2: Cytoplasmic Protein;
Lane 3: Nuclear Protein;
Lane 4: Membrane Protein;
Lane 5: Cytoskeleton Protein.

STORAGE/HANDLING:

Store Buffers C, W, N, M and CS at 2-8°C for up to 12 months after date of receipt.

Store the 50x PI Buffer at -20°C in undiluted aliquots for up to 12 months after date of receipt. Avoid repeated freeze/thaw cycles. Aliquot PI stock to minimize freeze-thaws.

Manufactured for Millipore by Biochain Institute, Inc.

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