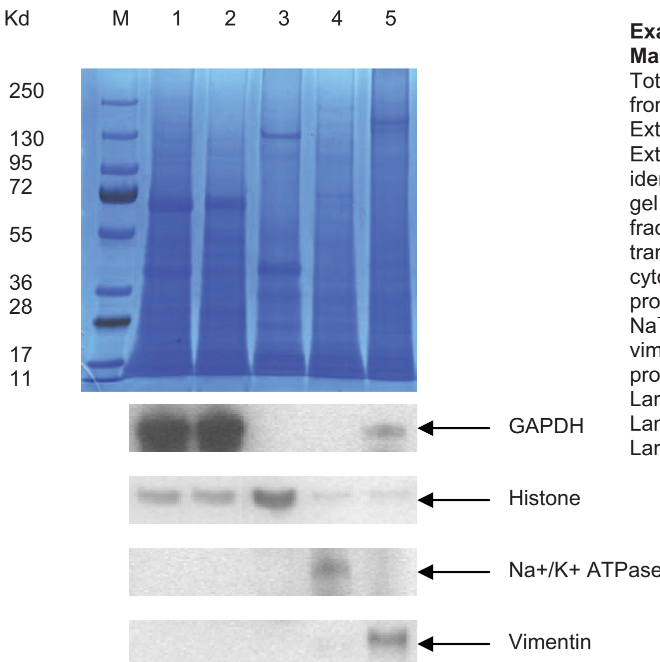
Compartmental Protein Extraction Kits

Extracting **CNMCS** (**C**ytoplasmic, **N**uclear, **M**embrane, and **C**ytoskeleton) proteins in one kit

Introduction

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.

The unique CNMCS and CNM Compartmental Protein Extraction Kits address 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. These unique kits can isolate four or three compartmental proteins. They are excellent tools for the initial purification and preparation of proteins for downstream applications including SDS-PAGE, Western blotting, gel mobility shift, protein assays and other procedures.



Example of CNMCS Protein Extraction from Mammalian Tissue
Total Protein and compartmental proteins extracted from rat colon tissue using amsbio's Total Protein Extraction Kit and CNMCS Compartmental Protein Extraction Kit were submitted to SDS-PAGE in 5 identical gels. Coomassie staining of one piece of gel indicated distinct protein pattern of respective fractions. Immunoblotting of PVDF membranes transferred from 5 other pieces of gel against cytoplasmic marker protein GAPDH, nuclear marker protein Histone H1, membrane marker protein Na^{+}/K^{+} ATPase, and cytoskeleton marker protein 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.

Features

- Convenient - Provides a complete set of components for stepwise preparation of cytoplasmic, nuclear, and membrane proteins from mammalian tissues and cultured cells
- Fast – isolate four or three compartmental proteins in less than three hours
- Reliable - Super-quality and highly reproducible separation of subcellular proteome fractions as verified by clearly distinct protein patterns and precise subcellular localizations of marker proteins
- Pure - Minimal cross contaminations
- Simple - Easy to use comparing to other methods, such as differential centrifugations

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

Description

Components in this kit are prepared with pure chemicals according to the proprietary technology. To prevent protein degradation, a ready-to-use protease inhibitor cocktail is provided. amsbio's Compartment Protein Extraction Kits are designed to sequentially isolate cytoplasmic, nuclear, membrane, and cytoskeleton proteins from mammalian tissues and cells. One Kit is consisted of reagents enough to enrich four or three compartmental proteins from 5 grams tissues or about 125 million cells. The efficiency of subcellular fractionation has been investigated by SDS-PAGE and immunoblotting of selected marker proteins.

Quality Control

One kit of this lot has been tested to go through the complete compartment protein extraction procedure from rat colon. Four compartmental proteins from the extraction are used for electrophoresis, transferring to PVDF membrane and immunoblotting with Mouse anti-GAPDH, Mouse anti-Histone H1, Mouse anti-Na^+/K^+ ATPase, and Mouse anti-Vimentin as primary antibodies, and HRP-conjugated anti-Mouse IgG as secondary antibody. The separation of four compartmental proteins is confirmed by the enrichment of GAPDH in the cytoplasmic fraction, Histone H1 in the nuclear fraction, Na^+/K^+ ATPase in the membrane fraction, and the unique localization of Vimentin in the cytoskeleton fraction.

Contents

CNMCS Kit

Item	Component	Amount	Part No.
Buffer C	HEPES (pH7.9), $MgCl_2$, KCl, EDTA, Sucrose, Glycerol, Sodium OrthoVanadate	18 ml	K3013010-1
Buffer W	HEPES (pH7.9), $MgCl_2$, KCl, EDTA, Sucrose, Glycerol, Sodium OrthoVanadate	50 ml	K3013010-2
Buffer N	HEPES (pH7.9), $MgCl_2$, NaCl, EDTA, Glycerol, Sodium OrthoVanadate	6 ml	K3013010-3
Buffer M	HEPES (pH7.9), $MgCl_2$, KCl, EDTA, Sucrose, Glycerol, Sodium deoxycholate, NP-40, Sodium OrthoVanadate	6 ml	K3013010-4
Buffer CS	Pipes (pH6.8), MgCl2, NaCl, EDTA, Sucrose, SDS, Sodium OrthoVanadate	3 ml	K3013010-5
50 x PI	A cocktail of protease inhibitors	0.66 ml	K3013010-6

CNM Kit

Item	Buffer C	Buffer W	Buffer N	Buffer M	50 x PI
Amount	12 ml	50 ml	6 ml	6 ml	0.5 ml
Part No.	K3012010-1	K3012010-2	K3012010-3	K3012010-4	K3012010-5

Reagents are sufficient for extraction of compartment proteins from 5 g tissue or about 125 million cells. Minimal 0.1 g tissue or 2.5 million cells should be required.

Storage and Stability

Buffer C, W, N, M, and CS are stored at 2-8 °C. The 50 x PI solution should be stored at -20 °C. If the kit is going to be used for multiple extractions, aliquot the PI solution properly before storing. The kit is stable for 1 year when handled properly.

Reagents and Equipments not provided

- Tissue Homogenizer
- Centrifuge and microcentifuge
- Rolling facility

Reference

1. Neelam Sharma-Walia et al *J. Virol.,* Apr 2004; 78: 4207 – 4223
2. Christian Korn et al *J. Biol. Chem.,* Nov 2004; 10.1074/jbc.M413035200

Protocol

I. Extracting compartment proteins extraction from tissues.

For buffer C, N, M, and CS, **add 50xPI to working concentration (1 x) before usage**.

1. Weigh certain amount of tissue (Wt gram), chop it to small pieces, then pipette ice cold buffer C at **2.0 ml per gram tissue.** Homogenize* the tissue at moderate speed (e.g., speed 4) for 20 sec. Let it stand on ice for a few seconds, repeat homogenization twice.
2. Rotate the mixture at 4˚C for 20 min. Spin at 18,000 g at 4˚C for 20 min. The cytoplasmic proteins are in the supernatant, take out and save in another tube.
3. Resuspend the pellet with Wt x 4 ml ice cold buffer W, rotate at 4˚C for 5' min. Spin at 18,000 g at 4˚C for 20 min. Drain the supernatant.
4. Add Wt x 1.0 ml ice cold buffer N to resuspend pellet from step 3, rotate at 4˚C for 20 min. Spin at 18,000 g at 4˚C for 20 min. The nuclear proteins** are in the supernatant, take out and save it in another tube.
5. Add Wt x 1.0 ml ice cold buffer M to resuspend pellet from step 4, rotate at 4˚C for 20 min. Spin at 18,000 g at 4˚C for 20 min. The membrane proteins are in the supernatant. Take out and save in another tube.
6. Pre-warm CS buffer at RT or 37 ˚C to make it clear. Add Wt x 0.5 ml buffer CS to resuspend pellet from step 5, rotate at room temperature for 20 min. Spin at 18,000 g at 4˚C for 20 min, save supernatant.
7. Resuspend the pellet from step 6 with Wt x 1.5 ml ice cold buffer C, rotate at 4˚C for 5 min. Spin at 18,000 g at 4˚C for 20 min, save supernatant.
8. Combine the supernatants from step 6 and 7. The cytoskeleton proteins are in the mixed solution.
9. Measure the protein concentrations of four fractions. Aliquot and label the proteins properly, store at –70˚C.

*** 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.**

**** 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 buffer C, N, M, and CS, **add 50xPI to working concentration (1 x) 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 the cells with buffer C well and rotate the mixture at 4˚C for 20 min.
2. 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. Then 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.
3. Resuspend the pellet with ice cold buffer W at **4.0 ml per 20 million cells**, rotate at 4˚C for 5 min. Spin at 15,000 g at 4˚C for 20 min. Drain the supernatant.

4. Add ice cold buffer N at **1.0 ml per 20 million cells** to resuspend pellet from step 3, rotate at 4˚C for 20 min. 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.
5. Add ice cold buffer M at **1.0 ml per 20 million cells** to resuspend pellet from step 4, rotate at 4˚C for 20 min. 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.
6. 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 5, rotate at room temperature for 20 min. Spin at 15,000 g at 4˚C for 20 min, save supernatant.
7. Measure the protein concentrations of four fractions. 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 3 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 condition was 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 condition or the cell disruption condition was too harsh, and the nuclear membrane was broken.

5. Protein degradations

Make sure PI was added to Buffers C, N, M, and CS before use

50 x PI was not aliquot, and lost activity when frozen-thawed for too many times.

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