Nuclear/Cytosol Fractionation Kit

(Protein Isolation and Extraction)

Catalog No. K6911

Introduction

Cell Biologics' Nuclear/Cytosolic Fractionation Kit provides a simple and quick tool that enables the separation nuclear extract from the cytoplasmic fraction of mammalian cells. The optimized reagents and procedure provide extraction, with high protein recovery and little/or no cross-contamination. The extracted protein fractions are functional and suitable for downstream assays such as transcriptional activity, RNA splicing, DNA footprinting, gel shift assays (EMSA), reporter assays, enzyme activity assays, and Western blotting. Each kit provides sufficient quantities to perform 20 preps (up to 5 x 10^6 cells each).

Storage Conditions:

-20°C

Shipping Conditions:

Ice pack

Authorized Uses of *Cell Biologics'* Products

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Isolating both cytoplasmic/membrane and nuclear proteins

Materials: Tissue, Tissue grinder, Cell culture plates, Cell scraper

Kit Contents:

- Buffers for 100 Assays:
  - Buffer H: 30 ml
  - Buffer A: 2 ml
  - Buffer C: 20 ml
- DTT (1M): 100 ul
- Protease Inhibitor Cocktail: 100 uL

Protocol: Fractionation of Membrane / Cytoplasmic and Nuclear Proteins Instruments

General Method:

For Cells:

1. Place plates on ice.
2. Wash 2X with cold PBS.
3. Add 1.0 ml PBS-EDTA and scrape cells. Transfer to a microcentrifuge tube.
4. Pellet at 3k rpm for 5 min at 4 °C. Then go to step 5

For Tissues:

1. Dissect the tissue of interest with clean tools, on ice preferably, and as quickly as possible to prevent degradation by proteases.
2. Place the tissue in round-bottom microcentrifuge tubes or Eppendorf tubes and immerse in liquid nitrogen to snap freeze. Store samples at -80°C for later use or keep on ice for immediate homogenization.
3. Homogenize tissues in a tissue homogenizer or grinder with ice cold PBS with proteinase inhibitor. Homogenize thoroughly and keep the sample on ice for 30 min. Centrifuge at 5000 rpm for 5 minutes, and discard the supernatants.
4. Add about 3-fold volume buffer H into the pellets. Alternatively, add 300 µl Buffer H to 1mg tissue. Then go to step 6.
5. Resuspend in cold buffer H (250-500~1).
6. Incubate on ice for 5 min.
7. Pellet at 1,000 rpm in swinging bucket rotor for 10 min to pellet nuclei.
8. Transfer the supernatant to a new tube and for best results, clear the supernatant at 14,000 rpm for 15 min and transfer to a new tube. This contains the cytoplasmic and membrane proteins.
9. Wash and resuspend pellet from step 7 in 500~1 of Buffer A.
10. Pellet at 1000 rpm in a swinging bucket rotor. Discard supernatant.
11. Add 4 volumes of Buffer C (for a more concentrated extract, use 2 volumes of 2X Buffer C).
12. Vortex 15 min at 4°C, start on high speed vortex to loosen pellet, then turn to medium.
13. Pellet at 14,000 rpm for 10 min at 4°C.
14. Transfer the supernatant to new tube. This contains the nuclear extract.