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Sequential Detergent and Salt-based Subcellular Fractionation of Human Neural Stem Cells

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We use this protocol and it's working

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Abstract

Sequential fractionation of neural stem cells was performed to obtain cytoplasmic, membrane, and nuclear protein fractions for validation of Imagestream, immunocytochemistry, and biotin-enrichment data showing intracellular localization of p32 in human neural stem cells.

Guidelines

14. Centrifuge again at 12,000 rcf for 15 minutes.
15. Collect the supernatant as the TX-100+ KCl-nuclear protein fraction.

Insoluble Protein Fraction:

16. Resuspend the final pellet in 20µL of 5X SDS-PAGE sample loading buffer (250mM Tris-HCl, pH 6.8, 10% SDS, 30% glycerol, 10mM DTT, 0.05% bromophenol blue).
17. Boil the sample for 5 minutes to denature proteins, preparing them for analysis.

Analysis:

18. Analyze 10µg of protein from each fraction using Western blot employing antibodies specific to compartment markers, and loading controls.

Materials

- Cold Dulbecco's Phosphate-Buffered Saline (DPBS without Ca^{2+}/Mg^{2+})
- Cell scraper
- PBS (0.1 mM Ca^{2+} and 1 mM Mg^{2+})
- Protease inhibitor (Roche Applied Science, cOmplete ULTRA, 05892970001)
- Phosphatase inhibitor (Roche Applied Science, PhosSTOP, 04906837001)
- 1 mL syringes
- 27.5 G needle
- 35Ga needle
- Lysis buffer (50mM Tris-HCl, pH 7.5, 100mM NaCl, 0.5% Triton X-100)
- 0.5M KCl
- 5X SDS-PAGE sample loading buffer (250mM Tris-HCl, pH 6.8, 10% SDS, 30% glycerol, 10mM DTT, 0.05% bromophenol blue)

Cell Detachment and Initial Preparation

1 Culture hNSC on poly-L-ornithine and laminin-coated 6-well plates in growth medium (GM).

2 Wash hNSC twice with cold Dulbecco's Phosphate-Buffered Saline without Ca^{2+}/Mg^{2+} (DPBS -/-).

3 Detach the cells using a cell scraper in 500 µL PBS (0.1 mM Ca^{2+} and 1 mM Mg^{2+}) supplemented with protease inhibitor (Roche Applied Science, cOmplete ULTRA, 05892970001) and phosphatase inhibitor (Roche Applied Science, PhosSTOP, 04906837001). Keep on ice to prevent protein degradation.

Mechanical Disruption

4 Homogenize the detached cells by passing them through a 1 mL syringe fitted with a 27.5 G needle, using 10 up-and-down strokes. This disrupts the plasma membrane, releasing cytoplasmic contents.

Cytoplasmic Fraction Collection

5 Incubate the homogenate on ice for 10 minutes to ensure complete lysis.

6 Centrifuge at 12,000 g for 15 minutes at 4°C. Ensure the centrifuge is pre-cooled.

7 Carefully collect the supernatant as the PBS-soluble cytoplasmic protein fraction.

Membrane/Structural Protein Fraction

8 Resuspend the remaining cell pellet in 500µL of lysis buffer containing 50mM Tris-HCl (pH 7.5), 100mM NaCl, 0.5% Triton X-100, and protease and phosphatase inhibitors. The Triton X-100 helps solubilize membrane proteins.

9 Use a 1 mL syringe fitted with a 35 G needle for further disruption, performing 10 additional up-and-down strokes.

10 Incubate on ice for 10 minutes followed by centrifugation at 12,000 g for 15 minutes at 4°C.

11 Collect the supernatant as the detergent (TX-100)-soluble membrane/subcellular structure protein fraction.

Nuclear and Caveolae Protein Fraction

12 Resuspend the remaining pellet in 200µL of lysis buffer with 0.5M KCl and protease and phosphatase inhibitors to extract nuclear proteins.

13 Incubate on ice for 10 minutes to allow salt to permeabilize the nuclear envelope.

14 Centrifuge again at 12,000 g for 15 minutes.

15 Collect the supernatant as the TX-100+ KCl-nuclear protein fraction.

Insoluble Protein Fraction

16 Resuspend the final pellet in 20µL of 5X SDS-PAGE sample loading buffer (250mM Tris-HCl, pH 6.8, 10% SDS, 30% glycerol, 10mM DTT, 0.05% bromophenol blue).

17 Boil the sample for 5 minutes to denature proteins, preparing them for analysis.

Analysis

18 Analyze 10µg of protein from each fraction using Western blot employing antibodies specific to compartment markers, and loading controls.