Jul 09, 2024

Microsomal membrane isolation

Forked from Microsomal membrane isolation from cell culture

DOI
dx.doi.org/10.17504/protocols.io.5qpvo3w7dv4o/v1

Rania Abou El Asrar[1,2], Peter Vangheluwe[1,2]

[1]KU Leuven; [2]Aligning Science Across Parkinson's (ASAP)

ASAP Collaborative Rese…

Rania Abou El Asrar
KU Leuven

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.5qpvo3w7dv4o/v1

Protocol Citation: Rania Abou El Asrar, Peter Vangheluwe 2024. Microsomal membrane isolation. **protocols.io https://dx.doi.org/10.17504/protocols.io.5qpvo3w7dv4o/v1**

Protocol status: Working
This protocol is currently being used to assess the activity of P5B-ATPases.

Created: December 06, 2023

Last Modified: July 09, 2024

Protocol Integer ID: 91888

Keywords: ASAPCRN

Funders Acknowledgement:
Aligning Science Across Parkinson's
Grant ID: ASAP-000458

Abstract

Microsomal membrane isolation

Harvest cells

1 Harvest cells by detachment with phosphate buffered saline (PBS, Sigma, D8537-500ml) + 0.2% EDTA.

2 Pellet cells by centrifugation (5 min, 400 g_{avg}, 4°C).

3 Wash cell pellet in phosphate buffered saline (PBS, Sigma, D8537-500ml). Repeat 2 times.

4 Pellet cells by centrifugation (5 min, 400 g_{avg}, 4°C).

Lyse cells

5 Resuspend pellet in hypnotic LIS buffer (10 mM Tris HCl pH 7.5, 0.5 mM $MgCl_2$, 1 mM DTT) supplemented with protease inhibitor (Sigmafast, Sigma, S8830-20TAB).

6 Incubate 15 min on ice.

7 Transfer the suspension to a dounce homogenizer and complete 60 up-and-down strokes.

8 Add an equal volume of 1 M buffer (0.5 M sucrose, 10 mM Tris HCl, pH 7.5, 40 µM $CaCl_2$, 1 mM DTT) supplemented with protease inhibitor.

9 Complete another 30 up-and-down strokes.

10 Centrifuge lysate (10min, 1000 g_{avg}, 4°C) to pellet and remove nuclear fraction.

11 Collect supernatant to continue isolation.

Isolate microsomal fraction

12 Centrifuge supernatant from previous step (20min, 15 000 g_{avg}, 4°C) to pellet the mitochondrial/lysosomal fraction.

13 Collect supernatant to continue isolation (pelleted mitochondrial/lysosomal fraction can be stored for future analysis).

14 Centrifuge supernatant from previous step (35 min, 140 000 g_{avg}, 4°C) to pellet the microsomal fraction.

15 Resuspend pellets in 0.25 M sucrose, 1 mM DTT and supplemented with protease inhibitors.

16 Snap freeze the resuspended mitochondrial/lysosomal and microsomal fractions in liquid nitrogen.

17 Store at -80 °C.