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1.1.1. Membrane protein separation

Elizabeth Fozo¹¹In-house protocol

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Works for me

This protocol is published without a DOI.

Eadewunm

ABSTRACT

Separation of membrane and cytoplasmic protein

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KEYWORDS

null, Barry Bruce lab, microcentrifuge, Separation of membrane

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GUIDELINES

Separation of membrane and cytoplasmic protein.

If necessary perform acetone precipitation of the protein following protocol.

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ABSTRACT

Separation of membrane and cytoplasmic protein

Separation of membrane and cytoplasmic protein

1 Using micro-centrifuge in the lab

- Spin 17,000 g for 30 minutes
 - Transfer supernatant (Cytoplasmic fraction), wash the pellet with cold PBS and resuspend in 100ul of PBS (membrane fraction)
- 2 Use Ultracentrifuge (100,000 g, 4°C)- Talk to *Barry Bruce Lab 226 Hesler Biology Building
- Transfer supernatant (cytoplasmic fraction), wash pellet with cold PBS and resuspend in 100ul of PBS (membrane fraction)
- 3 The separate inner and outer membrane(subcellular fractionation)
- Ultracentrifuge (100,000 g, 4°C, 45 minutes)- Talk to*Barry Bruce Lab 226 Hesler Biology Building
 - Transfer supernatant (cytoplasmic fraction), wash pellet with cold PBS
 - Resuspend the pellet in 200ul of 0.2% sodium sarcosinate. Mix properly.
 - Incubate at room temperature for 30 minutes
 - Centrifuge at 100,000 g, 4°C, 15 minutes), transfer supernatant (inner membrane protein), wash pellet with cold PBS, and resuspend in 100 ul of PBS.

If necessary perform acetone precipitation of the protein following protocol.

- 4 *Barry Bruce lab:
- Set up the time for ultracentrifugation (use the signup sheet next to the ultracentrifuge).
 - Use table top Optima™ Max Ultracentrifuge (35,000 RPM = ~104000g), Beckman Coulter with 55 TLA rotor
 - Have your samples balanced
 - Turn the power ON. Press DOOR to unlock the chamber door; slid the door open
 - Get the rotor 55 TLA from 4°C, load your samples and install the rotor
 - Close the door chamber (light on DOOR should go away), press SPEED and enter 35000, press TIME, and enter your run time, press ENTER and START.
 - After the run is complete, press DOOR to unlock the chamber door. Take out your samples. Clean the rotor if necessary and place it back at 4°C. Slid the door close and turn off the machine.