Microsome Isolation from Tissue

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[Abstract] This protocol details the extraction of microsomes from frozen tissue in order to further examine the protein-protein interactions occurring within the endoplasmic reticulum. This protocol was adapted from Abisambra *et al.* (2013) with modifications made in order to optimize for subsequent use.

Materials and Reagents

1. Sucrose
2. Protease Inhibitor cocktail, EDTA free (Merck KGaA, Calbiochem, catalog number: 539134)
3. Phosphatase inhibitor cocktail II
4. Phosphatase inhibitor cocktail III
5. PMSF at 10 mM in DMSO or 1.74 mg/ml (Thermo Fisher Scientific, catalog number: 36978)
6. Phosphatase Arrest II cocktail (Geno Technology, catalog number: 786-451)
7. Phosphatase Arrest III cocktail (Geno Technology, catalog number: 786-452)
8. M-PER Mammalian Protein Extraction Reagent (Thermo Fisher Scientific, catalog number: 78501)

Equipment

1. Sterile bottle filter
2. Glass Dounce homogenizer
3. Refrigerated centrifuge
4. Microfuge tubes rated for at least 25,000 *x g* centrifugation

Procedure

1. Make a 0.25 M sucrose solution that contains protease inhibitor cocktail, phosphatase inhibitor cocktails II and III, and PMSF as follows:
   Per 100 µl of Sucrose master mix add:
   a. 96 µl of 0.25 M sucrose
   b. 1 µl of protease inhibitor cocktail
   c. 1 µl of phosphatase inhibitor cocktail II
   d. 1 µl of phosphatase inhibitor cocktail III
   e. 1 µl of PMSF
2. Weigh tissue to be analyzed and add 10x its mass in volume of sucrose master mix (see step 1; *i.e.* 100 mg = 1,000 µl of sucrose solution).
3. While keeping all solutions on ice, add the appropriate amount of sucrose solution to tissue and dounce homogenize until a completely homogenous solution is obtained.
4. Spin the homogenate at 10,000 *x g* for 10 min at 4 °C.
5. Transfer the supernatants to a new microfuge tube (save the pellet at -20 °C) and spin at 30,000 *x g* for 90 min in a fixed angle rotor (or at 25,800 *x g* for 2 h).
6. Transfer the supernatant to a different microfuge tube and save at -20 °C. The remaining pellet corresponds to the microsomal fraction.
7. Pipette gently to resuspend the microsome pellet in 200 µl of the following mix (per 100 µl):
   a. 96 µl of MPER buffer
   b. 1 µl of protease inhibitor cocktail
   c. 1 µl of phosphatase arrest cocktail II
   d. 1 µl of phosphatase arrest cocktail III
   e. 1 µl of PMSF

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