

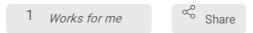


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Preparation of ATP13A2 microsomes from Sf9 cells

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ABSTRACT

Isolate microsomes from Sf9 cells expressing ATP13A2

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MATERIALS TEXT
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Phosphate-buffered Saline (ph 7.4) 137 mM NaCl 2.7 mM KCl 10 mM Na₂HPO₄ 1.8 mM KH₂PO₄

Lysis Buffer 10 mM Tris pH 7.5 0.5 mM MgCl2 2 mM DTT

0.1 mM PMSF

Plus protease inhibitors (5 μg/mL aprotinin, 5 μg/mL leupeptin, 1 μg/mL pepstatin A)

Resuspension Buffer 10 mM Tris pH 7.5 0.5 M sucrose 2 mM DTT 0.5 mM PMSF

Storage Buffer 0.25 M sucrose

Plus protease inhibitors (5 μ g/mL aprotinin, 5 μ g/mL leupeptin, 1 μ g/mL pepstatin A, 2 mM PMSF)

- 1 Thaw Sf9 cell pellets at room temperature (typical size around 5g for 0.4 L of culture)
- 2 All subsequent steps should be carried out at & 4 °C
- Wash pellet twice with 15 mL of Phosphate-buffered Saline, centrifuge at \$\mathbb{3} 1000 \text{ x g, 4°C, 00:07:00}\$ in between washes

7m

3.1 Gently resuspend by inverting tube and pipetting

7m

4 Collect cells after final wash by centrifugation at @1500 x g, 4°C, 00:07:00

⊗ proto	cols.io 3
15	Flash-freeze in aliquots of 25-100 μ L at concentrations between 2-5 mg/mL using liquid
14	Measure microsome concentrations based on total protein concentration using the Bradford Assay and bovine serum albumin as a standard
13	Resuspend microsomal pellet in Storage Buffer
12	Transfer supernatant to ultracentrifuge tubes and spin down at ©200000 x g, 4°C, 00:35:00 (Beckman Type 45 Ti rotor) to collect microsomes
11	Spin down at 310000 x g, 4°C, 00:20:00 (Sorvall SS-34 rotor) to remove mitochondriallysosomal fraction and save supernatant
10	Spin down at 31000 x g, 4°C, 00:10:00 to remove nuclear fraction and unbroken cells and save supernatant
9	Further lyse with Dounce homogenizer, 20 strokes tight
8	Dilute homogenate in equal volume Resuspension Buffer and mix
7	Lyse with Dounce homogenizer, 40 strokes tight
6	Swell cells in Lysis Buffer by incubating on ice for © 00:10:00 to © 00:15:00
5	Resuspend cells in 10 mL Lysis Buffer

nitrogen and store at -80C until use

