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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

Phosphate-buffered Saline (ph 7.4)
137 mM NaCl
2.7 mM KCl
10 mM Na_2HPO_4
1.8 mM KH_2PO_4

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 µg/mL aprotinin, 5 µg/mL leupeptin, 1 µg/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**

3 Wash pellet twice with 15 mL of Phosphate-buffered Saline, centrifuge at **1000 x g, 4°C, 00:07:00** in between washes 7m

3.1 Gently resuspend by inverting tube and pipetting

4 Collect cells after final wash by centrifugation at **1500 x g, 4°C, 00:07:00** 7m

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5 Resuspend cells in 10 mL Lysis Buffer

6 Swell cells in Lysis Buffer by incubating on ice for **00:10:00** to **00:15:00** 25m

7 Lyse with Dounce homogenizer, 40 strokes tight

8 Dilute homogenate in equal volume Resuspension Buffer and mix

9 Further lyse with Dounce homogenizer, 20 strokes tight

10 Spin down at **1000 x g, 4°C, 00:10:00** to remove nuclear fraction and unbroken cells and save supernatant 10m

11 Spin down at **10000 x g, 4°C, 00:20:00** (Sorvall SS-34 rotor) to remove mitochondrial-lysosomal fraction and save supernatant 20m

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 35m

13 Resuspend microsomal pellet in Storage Buffer

14 Measure microsome concentrations based on total protein concentration using the Bradford Assay and bovine serum albumin as a standard

15 Flash-freeze in aliquots of 25-100 µL at concentrations between 2-5 mg/mL using liquid

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nitrogen and store at -80C until use

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