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Purification of human ATP13A2 for cryo-EM analysis

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ABSTRACT

Purification of GFP-tagged human ATP13A2 expressed in Sf9 cells for cryo-EM analysis

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MATERIALS TEXT

Lysis Buffer
50 mM Tris pH 7.5
200 mM NaCl
1 mM EDTA
1 mM DTT
10% glycerol
Plus protease inhibitors (5 µg/mL aprotinin, 5 µg/mL leupeptin, 1 µg/mL pepstatin A, and 2 mM PMSF)

Wash Buffer
25 mM Tris pH 7.5
100 mM NaCl
1 mM EDTA
1 mM DTT
0.03% DDM/ 0.006% CHS

Day 1: Crude membrane preparation and solubilization 7h

1 Thaw Sf9 cell pellets at room temperature (typical size around 10 g from 0.7L of culture)

2 All subsequent steps performed at **4 °C**

3 Resuspend each pellet in 30 mL Lysis Buffer (use 3x volume of cell pellet, 40 mL total volume)

4 Homogenize pellet with Dounce homogenizer, 100 plunges tight on ice

5 Pour lysate into pre-chilled 50 mL centrifuge tubes

6 Spin in centrifuge at **4000 x g, 4°C, 00:10:00** to remove unbroken cells 10m

7 Transfer supernatant to pre-chilled ultracentrifuge rotor tubes

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8 Spin lysate in ultracentrifuge at **100000 x g, 4°C, 01:30:00** to pellet membranes (Beckman Type 45 Ti rotor) 1h 30m

9 Resuspend membrane pellet in Lysis Buffer and final volume 1% DDM/0.2% CHS (1X pellet, 7X Lysis Buffer, 2X 5% DDM/1% CHS)
DDM: n-dodecyl-β-D-maltopyranoside (Anatrace)
CHS: cholesteryl hemisuccinate (Anatrace)

10 Solubilize by rotating end-over-end for **02:30:00** at **4 °C** 2h 30m

11 Clarify lysate in ultracentrifuge at **100000 x g, 4°C, 01:00:00** 1h

Day 1: Bead binding and overnight 3C cleavage 3h

12 Equilibrate 1 mL Sepharose beads conjugated with anti-GFP nanobody with Wash Buffer

13 Add beads to gravity column and wash with 10 mL wash buffer

14 After ultracentrifugation is complete, transfer supernatant into 50 mL falcon tube

15 Add 1 mL equilibrated anti-GFP nanobody beads to tube

16 Incubate by rotating end-over-end for **02:30:00** at **4 °C** 2h 30m

17 Transfer to gravity column and let flow-through drain

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18 Wash beads with 30 column volumes of Wash Buffer

19 Add 5 mL Wash Buffer to gravity column and 10 µg/mL HRV 3C protease

20 Incubate by rotating end-over-end overnight at **4 °C**

Day 2: SEC column 3h

21 Equilibrate Superose 6 Increase 10/300 GL column with Wash Buffer

22 Concentrate the protein to 0.5 mL using an Amicon Ultrafilter (cut-off 100kDa)

23 After concentration, spin protein at **17000 x g, 4°C, 00:10:00** 10m

24 Injected sample into FPLC

25 Collect peak fractions and concentrate to approximately 5-7 mg/mL for cryo-EM grid preparation

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