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# Fluorescence size exclusion chromatography (FSEC) from ATP13A2 microsomes

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ABSTRACT

Using fluorescence size exclusion chromatography (FSEC) to analyze ATP13A2 expression in microsomes

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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)

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

1 Thaw 100 µg microsomes on ice

2 Resuspend microsomes in Lysis Buffer and final volume 1% DDM/0.2% CHS (1X pellet, 3X Lysis Buffer, 1X 5% DDM/1% CHS) at **4 °C**

2.1 DDM: n-dodecyl-β-D-maltopyranoside (Anatrace)

2.2 CHS: cholesteryl hemisuccinate (Anatrace)

3 Solubilize by rotating end-over-end for 2 h at 4C

4 Clarify lysate by spinning at **17000 x g, 4°C, 01:00:00** 1h

5 Equilibrate Superose 6 column, connected to an HPLC system, with Running Buffer



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6 Inject 85 uL of lysate into HPLC

7 Monitor elution of GFP-tagged ATP13A2 constructs using a fluorometer (λex= 475 nm; λem= 510 nm; with a fixed gain) connected to the system



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