Cell fractionation – SHSY5Y, hATP13A2

Pelleting cells

- Collect medium.
- Wash cells with PBS [without $CaCl_2$, $MgCl_2$].
- Incubate cells with TrypLE, 37°C.
- Collect cells.
- Centrifuge for 5 min at 300 g.
- Decant supernatant.
- Wash pellet with PBS.
- Centrifuge for 5 min at 300 g.
- Decant supernatant.
- Proceed with preparation of total cell lysate or microsomes.

Preparation of total cell lysate

- Add REPA lysis buffer (supplemented with protease inhibitors/phosphatase inhibitors).
- Incubate for 30 min (ON).
- Centrifuge for 30 min at 12,000 rpm (15,000 g) to pellet DNA.
- Keep supernatant.
- Determine concentration.

Preparation of microsomes

Cell lysis

- Resuspend pellet in 2 mL hypotonic LIS buffer.
- Leave cells on ice for 10-15 min to let them swell.
- Transfer to Dounce homogenizer and apply 40 up-and-down strokes.
- Add 2 mL of 1 M solution and apply another 20 up-and-down strokes.
- Transfer to Sarstedt tubes.

Differential centrifugation

- Centrifuge for 10 min at 1,000 g, 4°C to pellet the nuclear fraction.
- Transfer supernatant to a new Sarstedt tube.
- Centrifuge for 20 min at 9,000 rpm, 4°C to pellet the mitochondrial/lysosomal fraction.
- Resuspend pellet in 0.25 M sucrose (+ SF).
- Transfer supernatant to Beckman centrifuge tube.
- Equilibrate the tubes with 1 M solution.
- Ultracentrifuge for 35 min at 200,000 g (48,000 rpm), 4°C to pellet the microsomal fraction.
- Discard supernatant and clean the tubes with tissues.
- Resuspend pellet in 0.25 M sucrose (+ SF).
- Flash-freeze in liquid N_2.

Solutions

LIS buffer pH 7.5

- 10 mM Tris.HCl pH 7.5 [60.57 mg/50 mL]
- 0.5 mM $MgCl_2.6H_2O$ [5.0825 mg/50 mL]
- Protease inhibitors (*always add fresh*)

1 M solution

- 0.5 M sucrose
- 10 mM Tris.HCl pH 7.3
- 40 µM $CaCl_2$
- 0.23 mM PMSF (*always add fresh*)
- 1 mM DTT (*always add fresh*)