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Microsomal membrane isolation

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Protocol status: Working

This protocol is currently being used to assess the activity of P5B-ATPases.

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Abstract

Microsomal membrane isolation

Harvest cells

- 1 Harvest cells by detachment with phosphate buffered saline (PBS, Sigma, D8537-500ml) + 0.2% EDTA.
- 2 Pellet cells by centrifugation (5 min, 400 g_{avg} , 4°C).
- 3 Wash cell pellet in phosphate buffered saline (PBS, Sigma, D8537-500ml). Repeat 2 times.
- 4 Pellet cells by centrifugation (5 min, 400 g_{avg} , 4°C).

Lyse cells

- 5 Resuspend pellet in hypotonic LIS buffer (10 mM Tris HCl pH 7.5, 0.5 mM $MgCl_2$, 1 mM DTT) supplemented with protease inhibitor (Sigmafast, Sigma, S8830-20TAB).
- 6 Incubate 15 min on ice.
- 7 Transfer the suspension to a dounce homogenizer and complete 60 up-and-down strokes.
- 8 Add an equal volume of 1 M buffer (0.5 M sucrose, 10 mM Tris HCl, pH 7.5, 40 μM $CaCl_2$, 1 mM DTT) supplemented with protease inhibitor.
- 9 Complete another 30 up-and-down strokes.
- 10 Centrifuge lysate (10min, 1000 g_{avg} , 4°C) to pellet and remove nuclear fraction.
- 11 Collect supernatant to continue isolation.



Isolate microsomal fraction

- 12 Centrifuge supernatant from previous step (20min, 15 000 g_{avg}, 4°C) to pellet the mitochondrial/lysosomal fraction.
- 13 Collect supernatant to continue isolation (pelleted mitochondrial/lysosomal fraction can be stored for future analysis).
- 14 Centrifuge supernatant from previous step (35 min, 140 000 g_{avg}, 4°C) to pellet the microsomal fraction.
- 15 Resuspend pellets in 0.25 M sucrose, 1 mM DTT and supplemented with protease inhibitors.
- 16 Snap freeze the resuspended mitochondrial/lysosomal and microsomal fractions in liquid nitrogen.
- 17 Store at -80 °C.