Sucrose density gradient centrifugation of the post-mitochondrial fraction

Sample: crude membrane fraction (10 – 100 K, Ppt),

(+Mg): 10 mM Tris-acetate, pH 7.5, 5% (w/w) sucrose, 1 mM EGTA, 2 mM $MgCl_2$.

(+EDTA): 10 mM Tris-acetate, pH 7.5, 5%(w/w) sucrose, 1 mM EGTA, 2 mM EDTA.

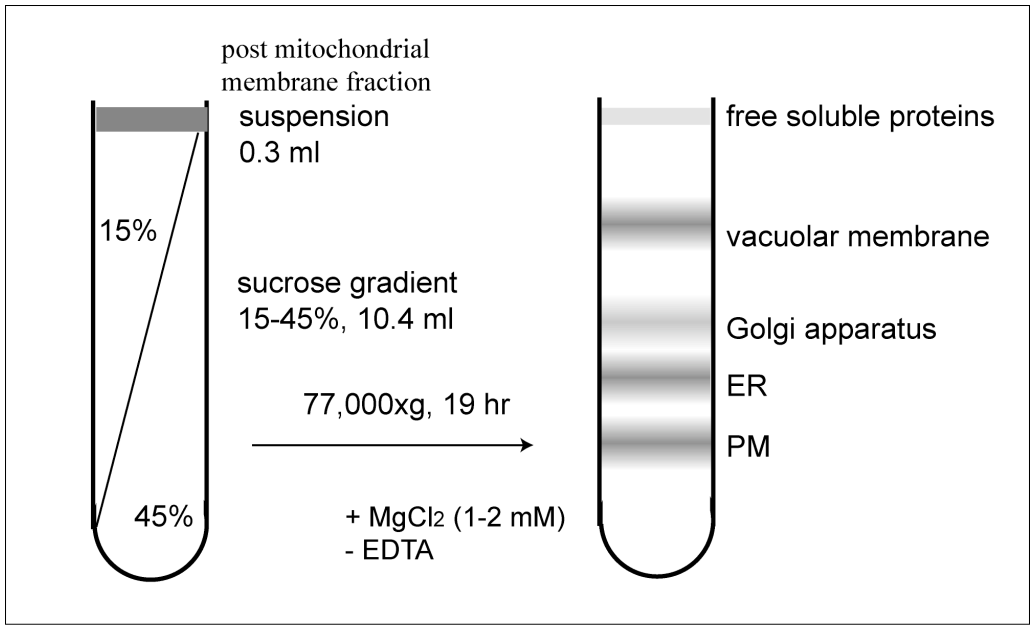
If you test the magnesium shift of the ER membranes, please prepare the both sample suspensions (+Mg and +EDTA), centrifuge and compare the distribution of ER marker proteins between the two tubes.

Sucrose gradients: 11-mL tube for ultracentrifugation.

15 – 45% (w/w) sucrose linear gradient (5.2 mL + 5.2 mL, total 10.4 mL)

Sucrose solution (+Mg): 10 mM Tris-acetate, pH 7.5, 1 mM EGTA, 2 mM $MgCl_2$

Sucrose solution (+EDTA): 10 mM Tris-acetate, pH 7.5, 1 mM EGTA, 2 mM EDTA



For analytical fractionation

Stock solution

0.5 M Tris-acetate, pH 7.5
100 mM Na-EGTA
100 mM MgCl2

Preparation of sucrose solutions

5% (w/w) sucrose / 10 mM Tris-acetate / 2 mM $MgCl_2$
15% (w/w) sucrose / 10 mM Tris-acetate / 2 mM $MgCl_2$
45% (w/w) sucrose / 10 mM Tris-acetate / 2 mM $MgCl_2$