Isolation of Plant Vacuolar Membranes

Homogenizing medium

0.25 M sorbitol	18.2	36.4	54.7 (g)
50 mM Tris/acetate (pH 7.5)*	0.5 M soln. × 40	80	120 (mL)
1 mM EGTA*	0.1 M soln. × 4	8	12 (mL)
20 μM APMSF[#]	2	4	6 (mg)
1% (w/v) PVP	4	8	12 (g)
2 mM DTT	123	247	370 (mg)
Final volume	400	800	1200 (mL)

[#] *p*-APMSF (Mr. 252.70): (*p*-amidinophenyl)methanesulfonyl fluoride hydrochloride (toxic)
APMSF should be added before experiment.

0.5 M Sucrose/Tris

0.5 M sucrose	18 g
20 mM Tris/acetate	0.5 M × 4 mL
1 mM EGTA	0.1 M × 1 mL
2 mM $MgCl_2$	1 M × 0.2 mL
2 mM DTT	31 mg
	100 mL

0.25 M Sorbitol/Tris

0.25 M sorbitol	4.55 g
20 mM Tris/acetate	0.5 M × 4 mL
1 mM EGTA	0.1 M × 1 mL
2 mM $MgCl_2$	1 M × 0.2 mL
2 mM DTT	31 mg
	100 mL

* 0.5 M Tris/acetate, pH 7.5 = 30.25 g / 500 mL
0.1 M EGTA = 19.0 g / 500 mL, adjust pH to 7.5 with KOH

Procedure

Plant Tissue (radish taproots or mung bean hypocotyls)
| ← homogenizing medium (chilled); [tissue : buffer = 1 : 1]
| homogenize by a grater (Oroshigane in Japanese)
| 7,000 rpm × 10 min
[Supernatant (Sup)]
| 40,000 rpm × 25 min (RP45T, Beckman 45Ti)
[Precipitate (Ppt)]
| ← suspend in 0.5 M Sucrose/Tris
| overlay with 0.25 M Sorbitol/Tris
| 40,000 rpm × 30 min (RP50T, Beckman 50.2Ti, slow brake)
[Interface between the two solutions]
| ← 0.25 M Sorbitol/Tris
| 40,000 rpm × 20 min
[Ppt]
| ← suspend in 0.25 M Sorbitol/Tris
| (or 20 mM Tris-acetate/ 20% glycerol/1 mM DTT/
| 1 mM EGTA/1 mM $MgCl_2$)
[Vacuolar membranes]