Preparation of Crude Membrane Fraction

Homogenizing medium

0.25 M sorbitol	18.2	(g)
50 mM Tris/acetate (pH 7.5)*	0.5 M × 40	(mL)
1 mM EGTA*	0.1 M × 4	(mL)
1 mM $MgCl_2$	0.1 M x 4	(mL)
(When you need to remove Mg^{2+}, please add EDTA instead of Mg^{2+})		
20 μM APMSF[#]	2	(mg)
1% (w/v) PVP	4	(g)
2 mM DTT	123	(mg)
Final volume	400	(mL)

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

5% (w/v) Sucrose/Tris

Sucrose	5.0 g
20 mM Tris/acetate	0.5 M x 4 mL
1 mM EGTA	0.1 M x 1 mL
2 mM $MgCl_2$	1 M x 0.2 mL
(When you need to remove Mg^{2+}, please add EDTA instead of Mg^{2+})	
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 or 1 : 2 (weight)]

| When you homogenize leaves, please add 5 to 10-fold volume of the buffer
| (10 g of leaves and 50 – 100 mL of buffer)

↓ homogenize with a Polytron

| 7,000 rpm × 10 min

Supernatant (Sup)

| 40,000 rpm × 25 min (RP45T, Beckman 45Ti)

Precipitate (Ppt)

| — suspend in 5% (w/v) Sucrose/Tris (or suitable buffer)

Crude membrane fractions

↓

Sucrose density gradient centrifugation (if you need)