Preparation of crude membrane fractions from rice tissues and organs

1) Solutions

Extraction buffer

50 mM KH_2PO_4-KOH, pH 7.5 at 4℃	Stock soln. 500 mM (1/10 dilution)
2 mM EGTA	Stock soln. 50 mM (1/25 dilution)
1 mM EDTA	Stock soln. 100 mM (1/100 dilution)
2 mM DTT	Conc. soln. 100 mM (1/50 dilution)
20 μM leupeptin	Conc. soln. 2 mM (1/100 dilution)
500 μM AEBSF	Conc. soln. 10 mM (1/20 dilution)
0.25 M D(-)-Mannitol	0.04554 g/mL
0.1 % (w/v) BSA	1 mg/mL

＊AEBSF: 4-(2-aminoethyl)-benzenesulfonyl fluoride, Sigma A8456, AEBSF is irreversible serine protease inhibitor with high water solubility.

D1 buffer

10 mM KH_2PO_4-KOH, pH 7.8 at 4℃	Stock soln. 500 mM (1/50 dilution)
1 mM DTT	Conc. soln. 100 mM (1/100 dilution)
0.25 M D-Sorbitol	0.04554 g/mL

Suspension buffer

25 mM Hepes-BTP, pH 7.2 at 4℃	Stock soln. 250 mM (1/10 dilution)
＊BTP: BisTris Propane	
1 mM DTT	Conc. soln. 100 mM (1/100 dilution)
500 μM AEBSF	Conc. soln. 10 mM (1/20 dilution)
0.25 M D-Sorbitol	0.04554 g/mL

2) Preparation of crude membrane fraction

On ice

One to 2 g f.w. of tissues or organs harvested.

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Add 1 to 2 ml of ice-cooled extraction buffer per 1 g f.w. of samples.
Homogenize by using a mortal and pestle.

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Centrifuge at 10,000 xg for 20 min at 4℃.
Collect the supernatant.

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Divide the supernatant into two Open Top Thick-walled polycarbonate tubes (Beckman; capacity: 2 ml) and fill up with the extraction buffer.
Set tubes on TLS-55 rotor (Beckman; Swing-type) pre-cooled at 4℃ after balanced.
Set the rotor on Optima TLX ultracentrifuge (Beckman).
Centrifuge at 156,000 xg (50,000 rpm) for 30 min at 4℃.

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Transfer the supernatant as the soluble protein fraction into the new tube.
Recover the precipitation as the crude membrane fraction.

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Add 1 mL of the D1 buffer to the precipitation.
Re-suspend the precipitation by pipetting.
Fill up with the D1 buffer.

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Re-centrifuge at 156,000 xg (50,000 rpm) for 30 min at 4℃.

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Recover the precipitation as the crude membrane fraction.

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Added 10 to 40 μL of the suspension buffer to the prep.
Re-suspend the precipitation by pipetting.

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Quantify protein amounts in soluble and crude membrane fractions by the Bradford method using BSA as a standard.

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Divide into aliquots.
Freeze quickly with liquid nitrogen and stored at -80℃ until used.