Isolation of crude mitochondria from Arabidopsis leaves (Keech et al., 2005)

Plant Materials

Arabidopsis plants must be grown under short-day conditions, as described in the literature, and harvested before the beginning of the light cycle.

Method A: to obtain crude mitochondria

Grind about 5 g of leaves. All equipment should be chilled at 4˚C and centrifuged at 4˚C.

1. Grind 5 g leaf tissue, using a mortar and pestle, in 20 ml grinding buffer A with a small amount (0.5 g) of quartz (fine granular, Merck).

2. Filter the homogenates through a nylon mesh (20 μm).

3. Centrifuge the extract at 2,500 *g* for 5 min in a Hitachi RPR20-2 rotor (4,800rpm ,4 ˚C) and centrifuge the resultant supernatant at 15,000 *g* for 15 min in an RPR20-2 rotor (11,500rpm ,4˚C).

4. Resuspend the pellet in ~200 μl of wash buffer A, using a small paintbrush, giving a final volume of approximately 400μl. [Option for respiratory activity measurements: add four percent of the final volume of a stock solution of protease inhibitor cocktail (Roche Applied Science)]. The yield of mitochondria obtained will be sufficient for at least six respiratory measurements.

5. Respiratory measurements

Take the necessary measurements in a final volume of *0.5* ml of mitochondria containing assay buffer. Malate (10 mM) and glutamate (1 mM), or glycine (10 mM) and NADH (1 mM) can be used as substrates to stimulate respiration, and 50 nmol ADP added when appropriate.

Grinding buffer A (100 ml):

0.3 M sucrose (mw 342.30)		10.269 g
60 mM TES-KOH, pH 8.0	1.0 M stock	6 ml(1.376g)
10 mM EDTA-2Na· $2H_2O$ (mw. 372.24)	0.5M stock	74.45 mg(2ml)
10 mM KH_2PO_4 (mw 136.09)		136.09 mg
25mM tetrasodium pyrophosphate (mw 446.06)		1.115 g
1 mM glycine (mw 75.07)		7.51 mg
1% (w/v) polyvinylpyrrolidone-40		1.0 g
1% (w/v) bovine serum albumin (BSA)		1.0 g
50 mM sodium ascorbate (mw 198.11)		990.55 mg

Plus 20 mM cysteine (mw 121.16, 242.32 mg) added just prior to grinding, and readjustment of the pH to 8.0 with 1 M KOH.

Wash buffer A (100 ml):

0.3 M sucrose (mw 342.30)		10.27 g
10 mM TES (mw 229.25)		229.25 mg
10 mM KH_2PO_4 (mw 136.09)		136.09 mg
2 mM EDTA-2Na· $2H_2O$ (mw. 372.24)	0.5 M stock	74.45 mg (0.4ml)

Adjust pH 7.5 with 1 M KOH

Assay buffer(50 ml):

0.3 M sucrose (mw 342.30)	5.13 g
10 mM TES (mw 229.25)	114.6 mg
10 mM KCl (mw 74.55)	37 mg
2 mM $MgSO_4$ (mw 246.48)	25 mg
5 mM KH_2PO_4 (mw 136.09)	34 mg
defatted BSA 0.1%,	50 mg

Adjust pH 7.5 with 1 M KOH.

Substrate stock solution:

	MW	Volume	weight
1M malate(100×)	134.09	1ml	134 mg
100mM glutamate(100×)	147.13	1ml	14.7 mg
1M glycine(100×)	75.07	1ml	75 mg
100mM NADH(100×)	664.43	0.5ml	33 mg
100mM ADP(20×)	427/80.7%=529	1 ml	52.9 mg

Reaction solution

	In 1ml reaction solution
mitochondria	50 μl
1M malate(100×)	10 μl
or 1M glycine(100×)	
100mM glutamate(100×)	10 μl
or 100mM NADH(100×)	
Assay buffer	920 μl
5mM ADP(1×)	10 μl

Reference: Keech,O., Dizengremel,P. and Gardestrom P. (2005) Preparation of leaf mitochondria from *Arabidopsis thaliana*. Physiol. Plant. **124:** 403-409.

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