

Isolation of leaf mitochondria

- 1) 20 – 60 g of leaves are cut to small pieces, and disrupted with a Polytron (Kinematica, Kriens, Switzerland) or with a mortar and pestle in 150–250 mL of cold extraction medium [0.3M sucrose, 25 mM tetra-sodium pyrophosphate, 10 mM KH_2PO_4 , 2 mM EDTA, 1 mM glycine, 1% (w/v) polyvinylpyrrolidone (PVP)-40, 1% (w/v) bovine serum albumin (BSA), 20 mM ascorbate, pH 7.5]. Please set middle speed and disrupt the leaves with 6-7 quick grinds, when you use a Polytron. When you use a mortar and pestle, please disrupt the leaves for at least 10 min.
- 2) The homogenate is filtered through four layers of 20 μm of nylon mesh, and centrifuged for 5 min at 1,100 x g at 4°C.
- 3) The supernatant is centrifuged for 20 min at 18,000 x g at 4°C, and the pellet is gently re-suspended in 200 mL of wash medium [0.3M sucrose, 10 mM *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES), 1 mM glycine, 0.1% (w/v) BSA, pH 7.5] with a brush, and centrifuged for 5 min at 1,100 x g at 4°C.
- 4) The supernatant is centrifuged for 20 min at 18,000 x g at 4°C, and the pellet is gently re-suspended in 10 mL of wash medium with the brush.
- 5) Aliquots of 5 mL are then layered over 27.5 mL of solution [0.3M sucrose, 10 mM TES, 1 mM glycine, 0.1% (w/v) BSA, 28% (v/v) and a linear gradient of 0–4.4% (w/v) PVP-40, pH 7.5] in a centrifuge tube, and centrifuged for 40 min at 40,000 x g at 4°C. The solutions with the linear gradient of 0–4.4% (w/v) PVP-40 are prepared before the disruption of leaves.
- 6) The mitochondria are found as a tight light yellow-brown band near the bottom of the tube. The mitochondrial fraction is removed with a vacuum suction and pastle pipette, and

diluted in 250 mL of wash medium and centrifuged at 31,000 x g for 15 min at 4°C.

7) The supernatant is removed with a vacuum suction, and this wash is repeated. The final mitochondrial pellet is re-suspended in approximately 1 mL of wash medium.

8) Mitochondrial integrity was monitored as described by Neuburger *et al.* (1982).

References

- Day, Neuburger & Douce (1985) Biochemical characterizations of chlorophyll-free mitochondria from pea leaves. *Australian Journal of Plant Physiology* **12**, 219–228.
- Neuburger M., Journet E.P., Bligny R., Carde J.P. & Douce R. (1982) Purification of plant mitochondria by isopycnic centrifugation in density gradients of Percoll. *Archives of Biochemistry and Biophysics* **217**, 312–323.
- Taylor, Day & Millar (2002) Environmental stress causes oxidative damage to plant mitochondria leading to inhibition of glycine decarboxylase. *Journal of Biological Chemistry* **277**, 42663–42668.