

**Chloroplast Purification** - Chloroplast proteins were extracted from fresh leaf samples as described previously (Kubis et al., 2008). Briefly, 20 g of fresh leaf tissue was cut into small pieces and homogenized with 200 ml of ice cooled chloroplast isolation buffer containing 0.3 M sorbitol, 5 mM MgCl<sub>2</sub>, 5 mM EGTA, 5 mM EDTA, 20 mM HEPES/KOH (pH 8.0), and 10 mM NaHCO<sub>3</sub> in a blender for 2-3 sec. The homogenate was filtered through two layers of Miracloth (Calbiochem-Novabiochem, San Diego, CA, USA). The filtrate was then centrifuged at 3000 ×g for 5 min at 4 °C and the supernatant was discarded. The pellets were then re-suspended with 1 ml of chloroplast isolation buffer with gentle shaking, and the intact chloroplasts were isolated on a preformed Percoll gradient through centrifugation at 7000 ×g for 10 min at 4 °C. The lower band containing the intact chloroplasts was carefully collected and washed with a buffer containing 50 mM HEPES/NaOH (pH 8.0), 3 mM MgSO<sub>4</sub>, and 0.3 M sorbitol. After centrifugation at 2000 ×g for 5 min at 4 °C, the pellet was collected and re-suspended with the solubilization buffer as above.

**Chloroplast Purity Measurement** - Equal amounts (25 µg) of protein sample were separated on 15% SDS-PAGE and then transferred onto a PVDF membrane. The blotted membrane was blocked for 1 h in TBS (20 mM Tris-HCl, pH 7.5, 500 mM NaCl) containing 3% (w/v) gelatin (Bio-Rad) and/or 5% non fat milk (Skim milk; Difco, MD, USA). The membrane was subsequently incubated with the polyclonal antibodies anti-ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) large subunit (Zhang and Komatsu, 2000), anti-RuBisCO activase (Zhang and Komatsu, 2000), anti-ascorbate peroxidase (APX) (kindly provided by Dr. K. Yukawa, NICS, Tsukuba, Japan), anti-elongation factor 1-beta (Yang et al., 2005), and pyruvate dehydrogenase kinase (PDK) (a kind gift from Prof. Douglas D. Randall, University of Missouri, Columbia, USA) at 1:5000 dilutions for 3 h at room temperature. Secondary antibodies were anti-mouse or anti-rabbit IgG with conjugated horseradish peroxidase (HRP; Bio-Rad). After incubation for 1 h with the appropriate HRP-conjugated secondary antibodies, the immunoblot signals were detected using HRP color development reagent (Bio-Rad) and/or an ECL plus western blotting detection kit (GE Healthcare, NJ, USA) following the manufacturer's protocols. Please see the publication (Ahsan et al., 2010) for the purity used in this database.

## References

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