Purification of plastids by Percoll gradient

Grinding buffer

0.33 M sorbitol
50 mM HEPES-KOH (pH 7.5)
2 mM EDTA
5 mM sodium ascorbate

Washing buffer

0.33 M sorbitol
50 mM HEPES-KOH (pH 7.5)

Percoll solution A

30% (v/v) Percoll
50 mM HEPES-KOH (pH 7.5)
2 mM EDTA
0.33 M sorbitol

Percoll solution B

80% (v/v) Percoll
50 mM HEPES-KOH (pH 7.5)
2 mM EDTA
0.33 M sorbitol

All operations are carried out at 4°C.

1. 20 g of pea leaves or stems are cut into small pieces and ground using a blender several times for 2-3 seconds in 100 ml (5 to 10 times volume of sample) of cold grinding buffer. Longer grinding increases the proportion of broken plastids.
2. The homogenate is rapidly filtered through 4 layers of Miracloth (Calbiochem 475855).
3. The filtrate is then centrifuged at 5,900 rpm for 30 sec (R9AF, Hitachi High-Technologies).
4. The supernatant is decanted and the pellet is gently resuspended in a small amount (2-3 ml) of grinding buffer with a brush.
5. The suspension is layered on the top of the Percoll solution, which

consists of 10 ml of Percoll solution A and 2.5 ml of Percoll solution B for 16 ml tube, and centrifuged for 15 min at 7,000 rpm for 15 min (P28S2, Hitachi High-Technologies).

6. The intact plastids are recovered as a band at the interface of the 30 % and 80 % Percoll layers. The upper part, which includes broken plastids, is aspirated and discarded.
7. The intact purified plastids are collected by pipette.
8. The plastid suspension is diluted with washing buffer (10 volumes to 1 volume of plastid suspension) and recovered as a pellet after centrifugation (7,000 x g for 1 min). To remove all Percoll, repeat the washing procedure once.