Purification of Intanct Chloroplasts from Arabidopsis T87 Cultured Cells

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Step1. Preparation of protoplasts from Arabidopsis T87

Collect T87 cells cultivated in 15L of JPL medium for 12 days by centrifugation (2,000g x five minutes).

Wash the cells three times with A buffer (3mM MES-KOH(pH5.7), 400mM mannitol, 7mM $CaCl_2$).

The wet weight of the T87 cells is ~120g.

Suspend the cells well in 500ml of A buffer containing 1% cellulase (Yakult-Onozuka R-10) and 0.2% macerozime (Yakult R-10).

Rotate the mixture in a one liter flask using a rotary shaker at 100rpm for five hours in the incubator at 25℃.

Check the reaction using a microscope, then collect the protoplasts by centrifugation (200g x five minutes) at 4℃.

Re-suspend the protoplasts mildly in A buffer and centrifuge them (200g x five minutes) at 4℃.

Suspend in 50ml of chilled B buffer (25mM HEPES-NaOH(pH7.6), 2mM EDTA, 400mM mannitol, 2mM sodium Isoascorbate).

Step2 Purification of Intact Chloroplasts from protoplasts

Prepare a few sets of 30 - 70% sucrose gradient tubes by the simple but highly reproducible method developed by Abe, S. and E. Davies (see the photos)

1) Overlay the six (or 20) milliliters of 30% sucrose solution on the equal amount of 70% sucrose solution in each of 14 (or 50) ml tube
2) Close the screw caps tightly, then carefully topple them over sideways.
3) Keep them for three hours at room temperature.

4) Raise the tubes slowly and keep them at 4℃.

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Seal three 500ml beakers with two-ply nylon mesh (20um) and keep on ice.

Load protoplast suspension into a 50ml disposable syringe.

Pass the suspension three times through the mesh (20um), drawing circles on the mesh with the pressured syringe.

Centrifuge the filtrate (250g x five minutes) at 4℃ for removal of nucleus and cell debris as precipitation.

Seal three 500ml beakers with two-ply nylon mesh (10um) and keep on ice.

Load the supernatant of protoplasts into a 50ml disposable syringe.

Pass the supernatant three times through the mesh (10um) as above.

Add percoll to the filtrate up to 15% (V/V).

Centrifuge (15,000g x 20min) the percoll solution at 4℃ to precipitate chloroplasts

Suspend the precipitate with B buffer and fill up to 20ml.

Apply two milliliters (or 10ml) of the suspension on each premade 30 to 70% sucrose gradient in 14 (or 50) ml tube

Centrifuge (1,800g x 40min) at 4℃ with a swing rotor.

Collect chloroplast layer by microsyringe (If two bands appear, intact chloroplasts will be enriched in the lower layer) (4,000g x five minutes).

Re-suspend the intact chloroplasts with B buffer, and centrifuge.

From the results of protein quantification using the BCA method, the final fraction should contain around 300mg chloroplast proteins.