Preparation of Vacuole from Arabidopsis Leaves

Solutions

Medium V; 25 mM Tris-MES (pH 7.0) and 0.45 M mannitol

2.5% Percoll in Medium P; 2.5% (v/v) Percoll, 25 mM Tris-MES (pH 5.5) and 0.45 M mannitol

20% Percoll in Medium P; 20% (v/v) Percoll, 25 mM Tris-MES (pH 5.5) and 0.45 M mannitol

10% Percoll in Medium V; 10% (v/v) Percoll, 25 mM Tris-MES (pH 7.0) and 0.45 M mannitol

0.2 M K_2HPO_4 (Prepare before use)

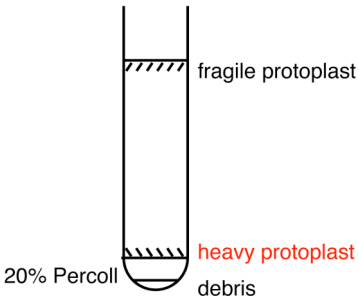
Enzyme solution; 1% (w/v) cellulase Onozuka-RS (Yakult, Tokyo, Japan), 0.1 % (w/v) pectolyase Y-23 (Seishin, Tokyo, Japan) in **2.5% Percoll in Medium P**. (Centrifuge before use to remove debris)

Procedure

Step1. Isolation of protoplasts

- Collect leaves in 50 ml beaker.
- Add 30 ml **Enzyme solution**.
- Vacuum infiltration.
- Gently shake for 40 min at room temperature.
- Filtrate the protoplasts with Miracloth.
- Collect the protoplasts in 15 ml test tubes.
- Add 0.5 ~ 1 ml of **20% Percoll in Medium P** to the bottom of the tubes.
- Centrifuge at 1,000 x *g* for 5 min.
- Collect heavy-protoplast fraction (Fig. 1).

Fig. 1



Step 2. Isolation of vacuoles from protoplasts

-Transfer 1 ml of heavy-protoplasts to new 15 ml test tube.

-Add 4 ml of **0.2 M K_2HPO_4**.

-Seal with Parafilm and gently mix by inverting the tube five times.

-Wait **1 min 45 sec**.

-Add 4 ml of **10% Percoll in Medium V**.

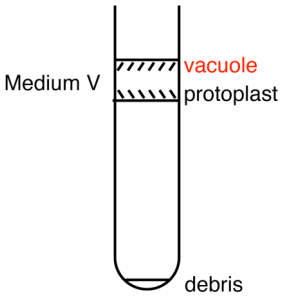
-Seal with Parafilm and gently mix by inverting the tube five times.

-Remove Parafilm and add 1~1.5 ml of **Medium V** to the top of mixture.

-Centrifuge at 800 x *g* for 5 min (slow acceleration, slow deceleration).

-Collect vacuole fraction (ca. 40 μl, Fig. 2).

Fig. 2



Notes

We can recover 5 ~ 10% vacuoles of total protoplasts with 85 ~ 95% purity.