Preparation of Vacuoplast and Miniplast from BY2 Cells

The vacuoplast is composed of a vacuole surrounded with a plasma membrane, while the miniplast is composed of a protoplast lacking vacuoles. You can determine the vacuolar-localization of the interested proteins by comparing vacuoplast and miniplast fraction Yamada et al (2001). The method is originally developed by Sonobe (1990).

Solutions

Medium P; 25 mM Tris-MES (pH 5.5) and 0.45 M mannitol

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

Enzyme solution; 1% (w/v) cellulase Onozuka-RS (Yakult, Tokyo, Japan), 0.1 % (w/v) pectolyase Y-23 (Seishin, Tokyo, Japan), 25 mM Tris-MES (pH 5.5) and 0.45 M mannitol. (Centrifuge before use to remove debris)

Percoll solution; 30 % (v/v) Percoll (Amersham Pharmacia Biotech), 25 mM Tris-MES (pH 7.0) and 0.45 M mannitol.

Procedure

-Set a Miracloth (Calbiochem) on grass funnel.

-Collect BY-2 cells on Miracloth. (6 day old, 40g)

-Wash the cells with Medium P (~30ml)

-Transfer the cells to two plastic dishes (ϕ = 9 cm), add 30 ml of enzyme solution (15 ml each).

-Keep 30°C for 2h

-Filtrate the protoplast with Miracloth.

-Dilute the protoplast solution with Medium P to ~1.5 fold of original volume.

-Separate the solution to two 50 ml Falcon tube.

-Centrifuge at 700 x *g* for 10 min

-Re-suspend the protoplast with 30 ml/tube of Medium V.

-Centrifuge at 700 x *g* for 10 min

-Re-suspend the protoplast with 7.5 ml/tube of Percoll solution.

-Transfer the solution to ultracentrifuge tube (we use ultra clear for SW28.1)

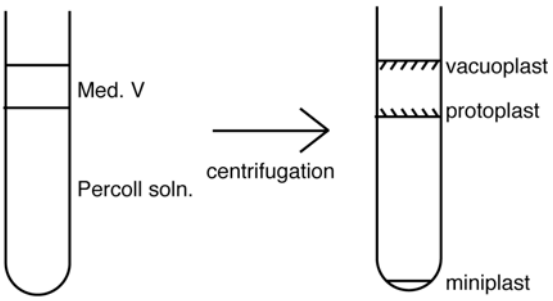
-Add 3 ml of Medium V on the top of Percoll solution (Fig. 1).

-Centrifuge at 10,000 x *g* for 1 h using a swing rotor (9,000 rpm with SW28.1, Beckman). Slow decelerating.

-The precipitate was composed of miniplasts. The upper fraction included vacuoplasts (Fig. 1).

-Recover each fraction.

Fig. 1



(Purification and concentration of the vacuoplast)

-Re-suspend the vacuoplasts with 10% Percoll solution (dilute Percoll solution with Medium V).

-Add 500 μl of Medium V on the top of Percoll solution.

-Centrifuge at 700 x *g* for 10 min.

-Recover the Medium V fraction.

(Purification and concentration of the miniplast)

-Add 5 ml of Medium V to miniplast fraction.

-Centrifuge at 700 x *g* for 10 min.

-Recover the precipitate.

Reference

Sonobe S

Cytochalasin B cytokinetic cleavage in miniprotoplast isolated from cultured tobacco cells. *Protoplasma* (1990) 155, 239-242.

Yamada K, Matsushima R, Nishimura M, Hara-Nishimura I

A slow maturation of a cysteine protease with a granulin domain in the vacuoles of senescing Arabidopsis leaves. *Plant Physiol*. (2001) 127, 1626-1634.