rpc00008

Arabidopsis thaliana T87 cell suspension culture

Components

• Domestic delivery: Two 50-mL tubes, containing 25 mL of cell suspension
• Overseas delivery: Two 250-mL flasks, containing cells placed on semi-solid medium

Notice

• Subculture the cells to fresh medium immediately after arrival [Notes I, II].
• Do not store the cell culture in a refrigerator and a freezer.
• Maintain aseptic conditions of the cell culture, and work in a laminar flow cabinet.

Summary

• Culture medium: JPL medium, 1 µM NAA (medium no. 5) [Materials III]
• Culture conditions: 22°C, continuous light, 120 rpm [Methods III]
• Subculture: 14-day intervals [Methods I]

Citation of cell line

When results obtained by using this cell line are published in a scientific journal, it should be cited in the following manner: “Arabidopsis thaliana T87 cell line (rpc00008) was provided by the RIKEN BRC through the National BioResource Project of the MEXT, Japan.”
Introduction

Arabidopsis T87 cell line was established from a seedling of Arabidopsis thaliana (L.) Heynh. accession Columbia (Axelos et al. 1992). The T87 cell culture is green and composed of small near-uniform aggregates of cells (Figure 1). The T87 cells are grown in a Jouanneau and Péaud-Lenoël (JPL) medium supplemented with 1 µM 1-naphthaleneacetic acid (NAA). Our T87 cell culture has been maintained under the continuous light at 22°C with rotary shaking at 120 rpm and subcultured at 14-day intervals (Figure 2).

Materials

I. Chemicals and stock solutions
(All stock solutions are stored at 4°C)

A) JPL_A'

KNO₃ 65.5 mg/mL
CaCl₂·2H₂O 4.4 mg/mL
MgSO₄·7H₂O 3.7 mg/mL
KH₂PO₄ 1.7 mg/mL

B) JPL_B

H₃BO₃ 6.2 mg/mL
MnSO₄·5H₂O 24.1 mg/mL
ZnSO₄·7H₂O 10.6 mg/mL
KI 0.83 mg/mL
Na₂MoO₄·2H₂O 0.25 mg/mL
CuSO₄ 5H₂O 0.025 mg/mL
CoCl₂·6H₂O 0.025 mg/mL

C) JPL_C

FeSO₄·7H₂O 2.78 mg/mL
Na₂·EDTA 3.73 mg/mL
Heat at 80°C for 3–4 hours for chelating Fe

D) JPL_D

Glycine 0.2 mg/mL
myo-Inositol 10 mg/mL

E) JPL_VT

Nicotinic acid 0.5 mg/mL
Pyridoxine·HCl 0.5 mg/mL
Thiamine·HCl 0.4 mg/mL

F) JPL_P
200 mM KH$_2$PO$_4$ 19.5 mL
200 mM Na$_2$HPO$_4$ 30.5 mL
H$_2$O 50 mL

G) NAA (1 mM)
NAA-K 0.224 mg/mL
Potassium 1-naphthylacetate, Wako Pure Chemical Industries (#161-04021)

H) Sucrose

I) Casein hydrolysate, vitamin free
Casamino acids vitamin assay, Difco (#228820)

J) KOH (1 N)
K) HCl (1 N)

II. Glassware and equipment
(All are sterilized by autoclaving at 121°C for 20 min)
A) Erlenmeyer flask (300 mL), capped with two layers of aluminum foil
B) Pipette (10 mL; large tip opening) and a bulb
C) Stainless sieve (diameter, 5 cm; pore size, 1 mm) set on a tall beaker (200 mL), capped with two layers of aluminum foil

III. Preparation of JPL medium (medium no. 5)
A) Prepare three solutions as follows.
A)-1 JPL mineral solution (1 L)
\[
\begin{align*}
\text{JPL}_A & \quad 37.5 \text{ mL} \\
\text{JPL}_B & \quad 0.375 \text{ mL} \\
\text{JPL}_C & \quad 2.5 \text{ mL}
\end{align*}
\]
Adjust the pH to 5.7 with KOH (1 N)
A)-2 JPL organic solution (100 mL)
\[
\begin{align*}
\text{JPL}_D & \quad 10 \text{ mL} \\
\text{JPL}_\text{VT} & \quad 1 \text{ mL} \\
\text{Casein hydrolysate} & \quad 0.1 \text{ g}
\end{align*}
\]
Adjust the pH to 5.7 with HCl (1 N)
A)-3 JPL sucrose solution (100 mL)
\[
\begin{align*}
\text{JPL}_P & \quad 1 \text{ mL} \\
\text{NAA (1 mM)} & \quad 1 \text{ mL} \\
\text{Sucrose} & \quad 15 \text{ g}
\end{align*}
\]
B) Autoclave the solutions at 121°C for 20 min.

C) Add 800 mL of JPL mineral solution, 100 mL of JPL organic solution, and 100 mL of JPL sucrose solution aseptically.

D) Pour 80 mL of the medium into a sterile 300-mL flask.

Methods

I. Filter a 14-day-old cell suspension through a stainless sieve (Figure 3).

II. Agitate the filtrate well and transfer 2.4 mL of cell suspension to 80 mL of fresh JPL medium with a pipette.

III. Incubate cell cultures on a rotary shaker at 120 rpm under the continuous light condition (photosynthetic photon flux density 40–42 µmol m$^{-2}$ s$^{-1}$) at 22°C.

Notes

I. For domestic customers: We send T87 cell suspension in 50-mL disposable tubes. The cells should be transferred to fresh JPL medium immediately after arrival. Transfer settled cells to Erlenmeyer flasks containing fresh liquid medium with a pipette.

II. For overseas customers: We send T87 cells placed on semi-solid JPL medium in 250-mL disposable flasks. The cells should be transferred to fresh JPL medium immediately after arrival. Collect the cells from the semi-solid medium with a spatula and transfer them to Erlenmeyer flasks containing fresh liquid medium.

III. In order to maintain T87 cell suspension cultures stably, it is essential to transfer an adequate amount of cells to fresh JPL medium in every subculture. The amount of cells may vary from one lab to another, because proliferation of T87 cells is affected by culture conditions, such as a room temperature, rotation speed of a rotary shaker, and aeration condition of the culture. T87-cell clumps occasionally develop into large aggregates, which causes a decrease in the amount of cells passed through a 1-mm sieve.

IV. In order to obtain good aeration of a suspension culture, a silicone sponge plug may be used instead of the aluminum foil cap (e.g., cap-type Silicosen; Shin-Etsu Polymer, Tokyo, Japan; https://www.shinpoly.co.jp/en/product/product/medical/plugs.html).

V. T87 cells may be maintained in Gamborg’s B5 medium (Yamada et al. 2004) or Murashige and Skoog medium (Cunillera et al. 2004) other than JPL
medium.

References


Figure 1. *Arabidopsis thaliana* T87 cell suspension culture

A: Two-week-old cell suspension culture.

B: Cell clumps. The size of cell clumps are varied, but most of them are below 1 mm. Scale bar = 1 mm

C: Morphology of T87 cells. T87 cells were observed by using differential interference contrast microscopy. The cells contain many chloroplasts. Scale bar = 50 µm
Figure 2. Growth profile of T87 cells
Figure 3. Procedure for subculturing T87 cells

A: Two-week-old cell suspension culture.

B: Pass the culture through a stainless sieve (pore size, 1 mm).

C: Transfer 2 mL of the filtrate to 80 mL of fresh JPL medium.
Appendix A: Formulation of culture medium

Table A1. Jouanneau and Péaud-Lenoël medium (medium no. 5)

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Concentration (mg/L)</th>
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<tbody>
<tr>
<td>KNO₃</td>
<td>1965</td>
</tr>
<tr>
<td>CaCl₂·2H₂O</td>
<td>132</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>111</td>
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<tr>
<td>KH₂PO₄</td>
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<tr>
<td>Na₂HPO₄</td>
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<tr>
<td>H₃BO₃</td>
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<tr>
<td>MnSO₄·5H₂O</td>
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<tr>
<td>ZnSO₄·7H₂O</td>
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<tr>
<td>KI</td>
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<tr>
<td>Na₂MoO₄·2H₂O</td>
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</tr>
<tr>
<td>CuSO₄·5H₂O</td>
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<tr>
<td>CoCl₂·6H₂O</td>
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<tr>
<td>FeSO₄·7H₂O</td>
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</tr>
<tr>
<td>Na₂-EDTA</td>
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<tr>
<td>Nicotinic acid</td>
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<tr>
<td>Pyridoxine·HCl</td>
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</tr>
<tr>
<td>Thiamine·HCl</td>
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<td>Glycine</td>
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<tr>
<td>myo-Inositol</td>
<td>100</td>
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<tr>
<td>Casein hydrolysate</td>
<td>100</td>
</tr>
<tr>
<td>Sucrose</td>
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<tr>
<td>NAA·K</td>
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