

rpc00008

***Arabidopsis thaliana* T87 cell suspension culture****Components**

- Domestic delivery: A 50-mL plastic conical centrifuge tube, containing cell suspension
- Overseas delivery: A 250-mL plastic Erlenmeyer flask, 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.

**Method**

- Culture medium: JPL medium, 1  $\mu$ M NAA (medium no. 5) [[Materials III](#)]
- Culture conditions: 22°C, continuous light, 120 rpm [[Methods II](#)]
- 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.”

Experimental Plant Division  
RIKEN BioResource Research Center (BRC)  
Koyadai 3-1-1, Tsukuba, Ibaraki 305-0074  
Japan  
FAX: +81 29 836 9053  
E-mail: [plant.brc@riken.jp](mailto:plant.brc@riken.jp)  
<http://epd.brc.riken.jp/en/>

## 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  $\mu$ M 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

### Chemicals and stock solutions

(All stock solutions are stored at 4°C)

#### A) JPL\_A'

KNO <sub>3</sub>	65.5 mg/mL
CaCl <sub>2</sub> ·2H <sub>2</sub> O	4.4 mg/mL
MgSO <sub>4</sub> ·7H <sub>2</sub> O	3.7 mg/mL
KH <sub>2</sub> PO <sub>4</sub>	1.7 mg/mL

#### B) JPL\_B

H <sub>3</sub> BO <sub>3</sub>	6.2 mg/mL
MnSO <sub>4</sub> ·5H <sub>2</sub> O	24.1 mg/mL
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	10.6 mg/mL
KI	0.83 mg/mL
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	0.25 mg/mL
CuSO <sub>4</sub> ·5H <sub>2</sub> O	0.025 mg/mL
CoCl <sub>2</sub> ·6H <sub>2</sub> O	0.025 mg/mL

#### C) JPL\_C

FeSO <sub>4</sub> ·7H <sub>2</sub> O	2.78 mg/mL
Na <sub>2</sub> -EDTA	3.73 mg/mL

Heat at 80°C for 3–4 hours for chelating Fe

#### D) JPL\_D

Glycine	0.2 mg/mL
<i>myo</i> -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 <sub>2</sub> PO <sub>4</sub>	19.5 mL
200 mM Na <sub>2</sub> HPO <sub>4</sub>	30.5 mL
H <sub>2</sub> O	50 mL

G) NAA (1 mM)

NAA·K	0.224 mg/mL
-------	-------------

Potassium 1-naphthylacetate, FUJIFILM Wako Pure Chemical Corporation (#161-04021)

H) Sucrose

I) Casein hydrolysate, vitamin free

Casamino acids vitamin assay, Difco (#228820)

J) KOH (1 N)

K) HCl (1 N)

**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

**Preparation of JPL medium (medium no. 5)**

1. Prepare three solutions as follows.

A) JPL mineral solution (1 L)

JPL_A'	37.5 mL
JPL_B	0.375 mL
JPL_C	2.5 mL

Adjust the pH to 5.7 with KOH (1 N)

B) JPL organic solution (100 mL)

JPL_D	10 mL
-------	-------

JPL_VT	1 mL
Casein hydrolysate	0.1 g
Adjust the pH to 5.7 with HCl (1 N)	

C) JPL sucrose solution (100 mL)

JPL_P	1 mL
NAA (1 mM)	1 mL
Sucrose	15 g

2. Autoclave the flask at 121°C for 20 min.
3. Add 800 mL of JPL mineral solution, 100 mL of JPL organic solution, and 100 mL of JPL sucrose solution aseptically.
4. Pour 80 mL of the medium into a sterile 300-mL flask.

## Methods

1. Filter a 14-day-old cell suspension through a stainless sieve (Figure 3).
2. Agitate the filtrate well and transfer 2–3 mL of cell suspension to 80 mL of fresh JPL medium with a pipette.
3. Incubate cell cultures on a rotary shaker at 120 rpm under the continuous light condition (photosynthetic photon flux density 40–42  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) at 22°C.

## Notes

- For domestic customers: We send T87 cell suspension in a 50-mL disposable conical centrifuge tube. The cells should be transferred to fresh JPL medium immediately after arrival.
- For overseas customers: We send T87 cells placed on semi-solid JPL medium in a 250-mL disposable Erlenmeyer flask. 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.
- 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.

- 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>).
- T87 cells may be maintained in Gamborg's B5 medium (Yamada *et al.* 2004) or Murashige and Skoog medium (Cunillera *et al.* 2000) other than JPL medium.

## References

- Axelos M, Curie C, Mazzolini L, Bardet C, Lescure B (1992) A protocol for transient gene expression in *Arabidopsis thaliana* protoplasts isolated from cell suspension cultures. *Plant Physiology and Biochemistry* 30: 123–128.
- Cunillera N, Arró M, Forés O, David Manzano, Ferrer A (2000) Characterization of dehydrolipichyl diphosphate synthase of *Arabidopsis thaliana*, a key enzyme in dolichol biosynthesis. *FEBS Letters* 477: 170–174. DOI: [10.1016/S0014-5793\(00\)01798-1](https://doi.org/10.1016/S0014-5793(00)01798-1)
- Yamada H, Koizumi N, Nakamichi N, Kiba T, Yamashino T, Mizuno T (2004) Rapid response of *Arabidopsis* T87 cultured cells to cytokinin through His-to-Asp phospho-relay signal transduction. *Bioscience, Biotechnology, and Biochemistry* 68: 1966–1976. DOI: [10.1271/bbb.68.1966](https://doi.org/10.1271/bbb.68.1966)

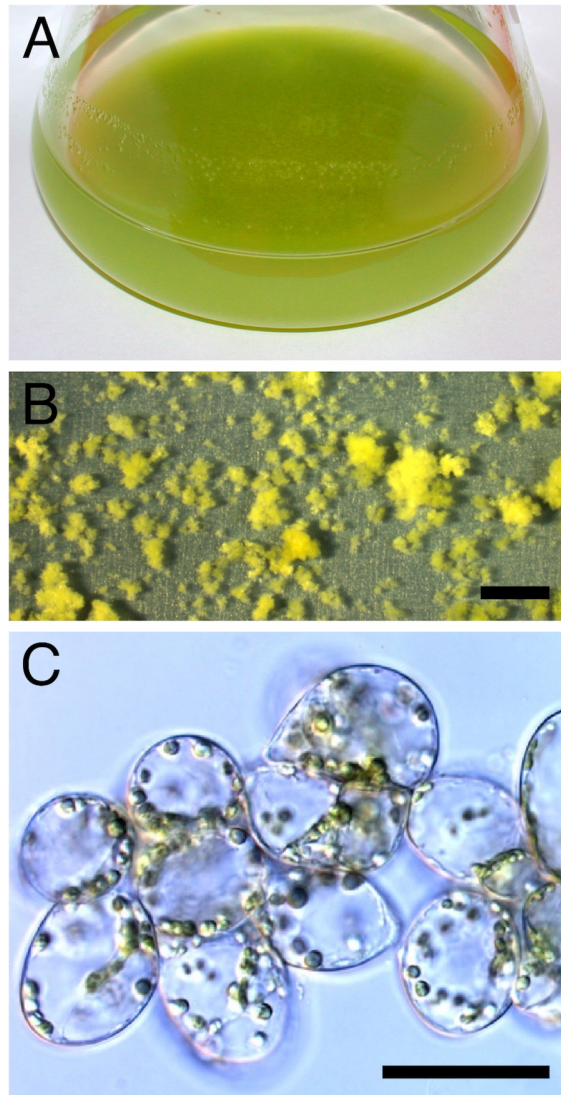


Figure 1. *Arabidopsis thaliana* T87 cell suspension culture

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  $\mu$ m

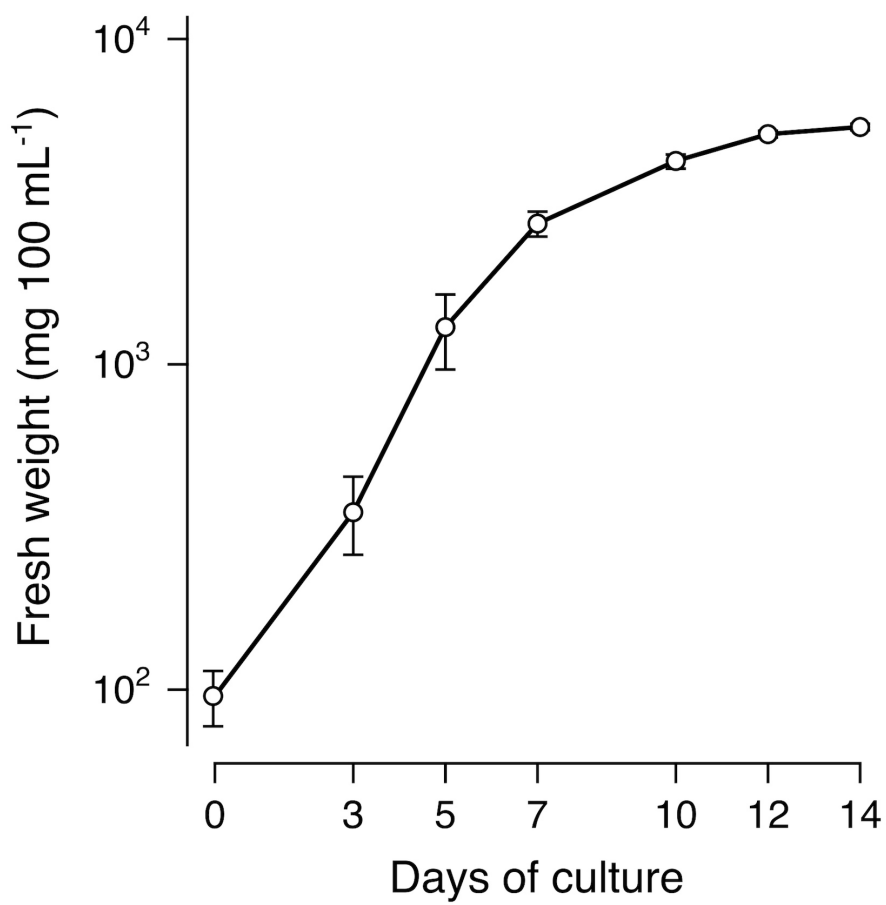


Figure 2. Growth profile of T87 cells

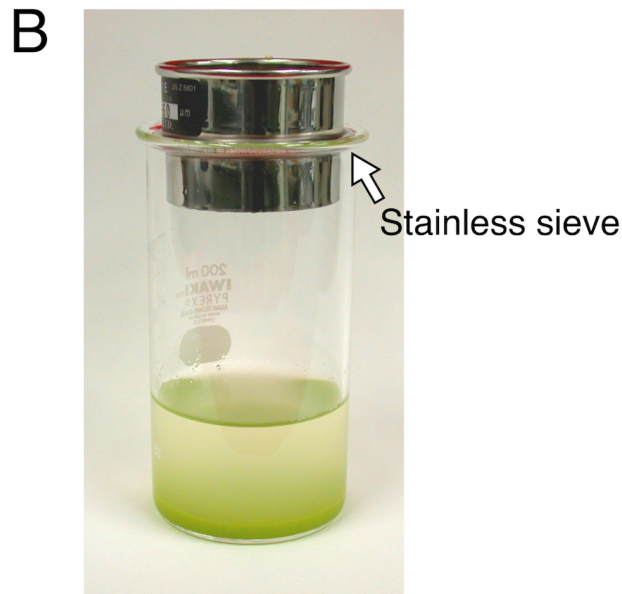
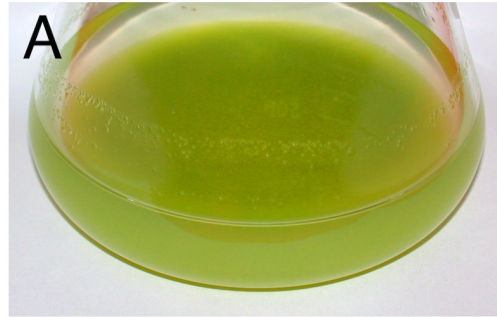


Figure 3. Procedure for subculturing T87 cells

- 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 A.1. Jouanneau and Péaud-Lenoël medium  
(medium no. 5)

Chemical	Concentration (mg/L)
KNO <sub>3</sub>	1965
CaCl <sub>2</sub> ·2H <sub>2</sub> O	132
MgSO <sub>4</sub> ·7H <sub>2</sub> O	111
KH <sub>2</sub> PO <sub>4</sub>	56.308
Na <sub>2</sub> HPO <sub>4</sub>	8.66
H <sub>3</sub> BO <sub>3</sub>	1.86
MnSO <sub>4</sub> ·5H <sub>2</sub> O	7.23
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	3.18
KI	0.249
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	0.075
CuSO <sub>4</sub> ·5H <sub>2</sub> O	0.0075
CoCl <sub>2</sub> ·6H <sub>2</sub> O	0.0075
FeSO <sub>4</sub> ·7H <sub>2</sub> O	5.56
Na <sub>2</sub> -EDTA	7.46
Nicotinic acid	0.5
Pyridoxine·HCl	0.5
Thiamine·HCl	0.4
Glycine	2
<i>myo</i> -Inositol	100
Casein hydrolysate, vitamin free	100
Sucrose	15000
NAA·K	0.224