

rpc00112

***Arabidopsis thaliana* At tom-c transgenic callus culture****Components**

- A 9-cm plastic Petri dish, containing cells placed on semi-solid medium

**Notice**

- Subculture the cells to fresh medium immediately after arrival [Notes I].
- 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: mLS medium, 0.2 mg/L 2,4-D, 0.4% (w/v) gellan gum, pH 5.8 (medium no. 71) [Materials III]
- Culture conditions: 27°C, dark [Methods II]
- Subculture: 14–21-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* At tom-c cell line (rpc00112) was provided by the RIKEN BRC through the National BioResource Project of the MEXT, Japan.”

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## Introduction

*Arabidopsis* At tom-c cell line was established by subculturing the At tom suspension culture on a semi-solid culture medium. The parent At tom cell line of *Arabidopsis thaliana* (L.) Heynh. accession Columbia a triple mutant that has *tom1-2* (ethyl methanesulfonate mutagenesis), *tom3-1* (ethyl methanesulfonate mutagenesis), and *thh1-1* (T-DNA insertion) mutations (Nishikiori *et al.* 2011). The At tom protoplasts show complete inhibition of tobamovirus multiplication. The At tom-c cells are grown on a modified Linsmaier and Skoog (mLS) medium supplemented with 0.2 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D), and solidified with 0.4% (w/v) gellan gum, pH 5.8. Our At tom-c cell culture has been maintained in the dark at 27°C and subcultured at 14–21-day intervals.

## Materials

### Chemicals and stock solutions

(All stock solutions are stored at 4°C)

A) MS salt mix

Murashige and Skoog Plant Salt Mixture, FUJIFILM Wako Pure Chemical Corporation (#392-00591)

B) Sucrose

C) BY2\_P

$\text{KH}_2\text{PO}_4$  80 mg/mL

D) LS\_VT\_modified

Thiamine·HCl 0.4 mg/mL  
*myo*-Inositol 40 mg/mL

E) 2,4-D (0.2 mg/mL)

2,4-D sodium monohydrate 0.236 mg/mL  
(2,4-Dichlorophenoxy)acetic acid sodium salt monohydrate, Sigma-Aldrich (D6679)

F) Gellan gum

G) KOH (1 N)

### Glassware and equipment

A) Petri dish (9 cm diameter, 2 cm height), sterile

B) Forceps, sterilized before use

C) Surgical tape

3M™ Micropore™ Surgical Tape, 12.5 mm × 9.1 m, 3M Japan Limited (#1530-0)

### Preparation of mLS medium (medium no. 71)

1. Dissolve the following chemicals in approximately 800 mL of distilled water.

MS salt mix	1 bag (1 L)
Sucrose	30 g

2. Add following stock solutions, and fill up to approximately 950 mL with distilled water.

BY2_P	2.5 mL
LS_VT_modified	2.5 mL
2,4-D (0.2 mg/mL)	1 mL

3. Adjust the pH of the solution to 5.8 with KOH (1 N), and fill up to 1 L with distilled water.

4. Add 4 g of gellan gum to the medium.

5. Autoclave the medium at 121°C for 20 min.

6. Pour 30 mL of the medium into a 9-cm Petri dish.

### Methods

1. Pick up an appropriate amount of callus cells from a 14–21-day-old culture with a forceps and place the cells onto fresh mLS medium.

2. Seal the Petri dishes using two rounds of surgical tape.

3. Incubate cell cultures under the dark condition at 27°C.

### Notes

- We send *At* tom-c cells on semi-solid mLS medium in a 9-cm disposable Petri dish. The cells should be subcultured to fresh mLS medium immediately after arrival.
- In order to maintain *At* tom-c callus culture stably, it is essential to observe the growth of cells carefully. Because proliferation of *At* tom-c cells is affected by culture conditions, such as a room temperature, aeration conditions of the culture and

so on, an amount of cells transferred to fresh medium and the subculture intervals may vary from one lab to another. We usually inoculate seven pieces of At tom-c callus (about 5-mm in diameter) on 30 mL of mLS medium in a 9-cm Petri dish, and culture them for 14–21 days.

- Suspension cultures can be derived from YU-1-c callus cultures by subculturing them into a liquid culture medium. The suspension culture method is the same as rpc00056 At tom.

## References

Nishikiori M, Mori M, Dohi K, Okamura H, Katoh E, Naito S, Meshi T, Ishikawa M (2011) A host small GTP-binding protein ARL8 plays crucial roles in tobamovirus RNA replication. PLoS Pathogen 7: e1002409. DOI: [10.1371/journal.ppat.1002409](https://doi.org/10.1371/journal.ppat.1002409)

## Appendix A: Formulation of culture medium

Table A.1. modified Linsmaier and Skoog medium  
(medium no. 71)

Chemical	Concentration (mg/L)
KNO <sub>3</sub>	1900
NH <sub>4</sub> NO <sub>3</sub>	1650
CaCl <sub>2</sub> ·2H <sub>2</sub> O	440
MgSO <sub>4</sub> ·7H <sub>2</sub> O	370
KH <sub>2</sub> PO <sub>4</sub>	370
H <sub>3</sub> BO <sub>3</sub>	6.2
MnSO <sub>4</sub> ·4H <sub>2</sub> O	22.3
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	8.6
KI	0.83
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	0.25
CuSO <sub>4</sub> ·5H <sub>2</sub> O	0.025
CoCl <sub>2</sub> ·6H <sub>2</sub> O	0.025
FeSO <sub>4</sub> ·7H <sub>2</sub> O	27.8
Na <sub>2</sub> -EDTA	37.3
Thiamine·HCl	1
<i>myo</i> -Inositol	100
Sucrose	30000
2,4-D sodium monohydrate	0.236
Gellan gum	4000