

rpc00050

## ***Arabidopsis thaliana* YG1 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: mLS medium, 0.2 mg/L 2,4-D, pH 5.8 (medium no. 1) [[Materials III](#)]
- Culture conditions: 27°C, dark, 130 rpm [[Methods II](#)]
- Subculture: 7-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* YG1 cell line (rpc00050) was provided by the RIKEN BRC through the National BioResource Project of the MEXT, Japan.”

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## Introduction

*Arabidopsis* YG1 cell line was established from a seedling of *Arabidopsis thaliana* (L.) Heynh. accession Columbia (Nishikiori *et al.* 2011, Yoshikawa *et al.* 2021). The YG1 cells are grown in a modified Linsmaier and Skoog (mLS) medium supplemented with 0.2 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D), pH 5.8. Our YG1 cell culture has been maintained in the dark at 27°C with rotary shaking at 130 rpm and subcultured at 7-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) KOH (1 N)

### Glassware

A) Erlenmeyer flask (300 mL), capped with two layers of aluminum foil

B) Pipette (10 mL; large tip opening) and a bulb, sterilized by autoclaving at 121°C for 20 min

### Preparation of mLS medium (medium no. 1)

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. Pour 80 mL of the medium into a 300-mL flask.
5. Autoclave the flask at 121°C for 20 min.

### Methods

1. Leave to stand a flask containing a 7-day-old culture until the cells settle to the bottom of the flask.
2. Transfer 4–6 mL of the sedimented cells to 80 mL of fresh mLS medium with a pipette.
3. Incubate cell cultures on a rotary shaker at 130 rpm under the dark condition at 27°C.

### Notes

- For domestic customers: We send YG1 cell suspension in a 50-mL disposable conical centrifuge tube. The cells should be transferred to fresh mLS medium immediately after arrival. Transfer settled cells to Erlenmeyer flasks containing fresh liquid medium with a pipette.
- For overseas customers: We send YG1 cells placed on semi-solid mLS medium in a 250-mL disposable Erlenmeyer flask. The cells should be transferred to fresh mLS 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 YG1 cell suspension cultures stably, it is essential to transfer an adequate amount of cells to fresh mLS medium in every subculture. The amount of

cells may vary from one lab to another, because proliferation of YG1 cells is affected by culture conditions, such as a room temperature, rotation speed of a rotary shaker, and aeration condition of the culture.

- 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>).

## 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)
- Yoshikawa M, Han Y-W, Fujii H, Aizawa S, Nishino T, Ishikawa M (2021) Cooperative recruitment of RDR6 by SGS3 and SDE5 during small interfering RNA amplification in *Arabidopsis*. Proceedings of National Academy of Sciences of USA 118: e2102885118. DOI: [10.1073/pnas.2102885118](https://doi.org/10.1073/pnas.2102885118)

## Appendix A: Formulation of culture medium

Table A1. modified Linsmaier and Skoog medium  
(medium no. 1)

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