

rpc00104

***Arabidopsis thaliana* MM2d-LS 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* MM2d-LS cell line (rpc00104) was provided by the RIKEN BRC through the National BioResource Project of the MEXT, Japan.”

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## Introduction

*Arabidopsis* MM2d-LS cell line was established by subculturing the MM2d cells in a different culture medium (Adachi *et al.* 2011). The parent MM2d cell line (rpc00103) was established from a stem of *Arabidopsis thaliana* (L.) Heynh. accession Landsberg *erecta* (Menges and Murray 2002). The MM2d-LS cell line can be used for cell cycle synchronization and genetic transformation (Adachi *et al.* 2011). The MM2d-LS 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 MM2d-LS 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

KH<sub>2</sub>PO<sub>4</sub> 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 50 mL of the medium into a 300-mL flask.
5. Autoclave the flask at 121°C for 20 min.

### Methods

1. Agitate a 7-day-old culture well and transfer 1–2 mL of cell suspension to 50 mL of fresh mLS medium with a pipette.
2. Incubate cell cultures on a rotary shaker at 130 rpm under the dark condition at 27°C.

### Notes

- For domestic customers: We send MM2d-LS cell suspension in a 50-mL disposable conical centrifuge tube. The cells should be transferred to fresh mLS medium immediately after arrival.
- For overseas customers: We send MM2d-LS 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 MM2d-LS 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 MM2d-LS 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

- Adachi S, Minamisawa K, Okushima Y, Inagaki S, Yoshiyama K, Kondou Y, Kaminuma E, Kawashima M, Toyoda T, Matsui M, Kurihara D, Matsunaga S, Umeda M (2011) Programmed induction of endoreduplication by DNA double-strand breaks in *Arabidopsis*. *Proceedings of National Academy of Sciences of USA* 108: 10004–10009. DOI: [10.1073/pnas.1103584108](https://doi.org/10.1073/pnas.1103584108)
- Menges M, Murray JAH (2002) Synchronous *Arabidopsis* suspension cultures for analysis of cell-cycle gene activity. *Plant Journal* 30: 203–212. DOI: [10.1046/j.1365-3113.2002.01274.x](https://doi.org/10.1046/j.1365-3113.2002.01274.x)

## Appendix A: Formulation of culture medium

Table A.1. 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