rpc00103

Arabidopsis thaliana MM2d 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: MS medium, 0.5 mg/L NAA, 0.05 mg/L kinetin, pH 5.8 (medium no. 61) [Materials III]
- Culture conditions: 27℃, 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 cell line (rpc00103) was provided by the RIKEN BRC through the National BioResource Project of the MEXT, Japan."

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Introduction

Arabidopsis MM2d cell line was established from a stem of *Arabidopsis thaliana* (L.) Heynh. accession Landsberg *erecta* (Menges and Murray 2002). The MM2d cell line can be used for cell cycle synchronization and genetic transformation (Menges and Murray 2002, Kono *et al.* 2006). The MM2d cells are grown in a Murashige and Skoog (MS) medium supplemented with 0.5 mg/L 1-naphthaleneacetic acid (NAA) and 0.05 mg/L kinetin, pH 5.8. Our MM2d cell culture has been maintained in the dark at 27℃ with rotary shaking at 130 rpm and subcultured at 7-day intervals.

Materials

Chemicals and stock solutions

(All stock solutions are stored at 4℃)

A) MS salt mix

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

B) Sucrose

C) MS_VT

Nicotinic acid	0.5 mg/mL
Pyridoxine·HCl	0.5 mg/mL
Thiamine·HCl	0.1 mg/mL
Glycine	2 mg/mL

D) MS_inositol

myo-Inositol	40 mg/mL

E) NAA (0.5 mg/mL)

NAA·K	0.6 mg/mL

Potassium 1-naphthylacetate, FUJIFILM Wako Pure Chemical Corporation (#161-04021); Sterilize the solution by membrane filtration, and store at −20℃

F) Kinetin (0.05 mg/mL)

Kinetin	0.05 mg/mL

Dissolve kinetin in small volume of KOH (1 N), and fill up with distilled water; Sterilize the solution by membrane filtration, and store at −20℃

G) 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℃ for 20 min

Preparation of MS medium (medium no. 61)

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.

MS_VT	1 mL
MS_inositol	2.5 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℃ for 20 min.

6. Add the following phytohormones to the medium just before subculturing.

NAA (0.5 mg/mL)	50 µL
Kinetin (0.05 mg/mL)	50 µL

Methods

1. Agitate a 7-day-old culture well and transfer 2 mL of cell suspension to 50 mL of fresh MS medium with a pipette.

2. Incubate cell cultures on a rotary shaker at 130 rpm under the dark condition at 27℃.

Notes

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

- For overseas customers: We send MM2d cells placed on semi-solid MS medium in a 250-mL disposable Erlenmeyer flask. The cells should be transferred to fresh MS

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 cell suspension cultures stably, it is essential to transfer an adequate amount of cells to fresh MS medium in every subculture. The amount of cells may vary from one lab to another, because proliferation of MM2d 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

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.1365-313X.2002.01274.x

Kono A, Ohno R, Umeda-Hara C, Uchimiya H, Umeda M (2006) A distinct type of cyclin D, CYCD4;2, involved in the activation of cell division in *Arabidopsis*. Plant Cell Reports 25: 540–545. PMID: 16408177. DOI: 10.1007/s00299-005-0075-4

Appendix A: Formulation of culture medium

Table A.1. Murashige and Skoog medium (medium no. 61)

Chemical	Concentration (mg/L)
KNO_3	1900
NH_4NO_3	1650
$CaCl_2 \cdot 2H_2O$	440
$MgSO_4 \cdot 7H_2O$	370
KH_2PO_4	170
H_3BO_3	6.2
$MnSO_4 \cdot 4H_2O$	22.3
$ZnSO_4 \cdot 7H_2O$	8.6
KI	0.83
$Na_2MoO_4 \cdot 2H_2O$	0.25
$CuSO_4 \cdot 5H_2O$	0.025
$CoCl_2 \cdot 6H_2O$	0.025
$FeSO_4 \cdot 7H_2O$	27.8
Na_2-EDTA	37.3
Nicotinic acid	0.5
Pyridoxine·HCl	0.5
Thiamine·HCl	0.1
Glycine	2
myo-Inositol	100
Sucrose	30000
NAA·K	0.6
Kinetin	0.05