

rpc00032

***Lotus japonicus* Lj 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, 1 mg/L 2,4-D, pH 5.7 (medium no. 27) [Materials III]
- Culture conditions: 27°C, dark, 120 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: “*Lotus japonicus* Lj cell line (rpc00032) was provided by the RIKEN BRC through the National BioResource Project of the MEXT, Japan.”

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Introduction

Lj cell line was established from a shoot apex of a seedling of *Lotus japonicus* (Regel) K.Larsen accession Gifu B-129. The Lj cells are grown in a Murashige and Skoog (MS) medium supplemented with 1 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D), pH 5.7. Our Lj cell culture has been maintained in the dark at 27°C with rotary shaking at 120 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) 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

<i>myo</i> -Inositol	40 mg/mL
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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 MS medium (medium no. 27)

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
2,4-D (0.2 mg/mL)	5 mL

3. Adjust the pH of the solution to 5.7 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 10 mL of the sedimented cells to 80 mL of fresh MS medium with a pipette.
3. Incubate cell cultures on a rotary shaker at 120 rpm under the dark condition at 27°C.

Notes

- For domestic customers: We send Lj cell suspension in a 50-mL disposable conical centrifuge tube. The cells should be transferred to fresh MS medium immediately after arrival. Transfer settled cells to Erlenmeyer flasks containing fresh liquid medium with a pipette.
- For overseas customers: We send Lj 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 Lj 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 Lj 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>).
- The parent accession Gifu B-129 is available from National BioResource Project Lotus/Glycine (<https://www.legumebase.brc.miyazaki-u.ac.jp/>).

Appendix A: Formulation of culture medium

Table A1. Murashige and Skoog medium
(medium no. 27)

Chemical	Concentration (mg/L)
KNO ₃	1900
NH ₄ NO ₃	1650
CaCl ₂ ·2H ₂ O	440
MgSO ₄ ·7H ₂ O	370
KH ₂ PO ₄	170
H ₃ BO ₃	6.2
MnSO ₄ ·4H ₂ O	22.3
ZnSO ₄ ·7H ₂ O	8.6
KI	0.83
Na ₂ MoO ₄ ·2H ₂ O	0.25
CuSO ₄ ·5H ₂ O	0.025
CoCl ₂ ·6H ₂ O	0.025
FeSO ₄ ·7H ₂ O	27.8
Na ₂ -EDTA	37.3
Nicotinic acid	0.5
Pyridoxine·HCl	0.5
Thiamine·HCl	0.1
Glycine	2
<i>myo</i> -Inositol	100
Sucrose	30000
2,4-D sodium monohydrate	1.18