rpc00002

Daucus carota kurodagosun 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: modified Lin and Staba medium, 0.2 mg/L 2,4-D, pH 5.7 (medium no. 67) [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: "*Daucus carota* kurodagosun cell line (rpc00002) was provided by the RIKEN BRC through the National BioResource Project of the MEXT, Japan."

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Introduction

Carrot kurodagosun cell line is an embryogenic cell culture induced from a hypocotyl of *Daucus carota* L. cultivar Kurodagosun according to the method described by Fujimura and Komamine (1979). The cell clusters proliferate as undifferentiated cells in the presence of 2,4-dichlorophenoxyacetic acid (2,4-D), but develop into somatic embryos by transferring them to phytohormone-free medium. Because the embryogenic competence decreases during repeated subculturing, we have been establishing the kurodagosun cell culture at about 6-month intervals. The kurodagosun cells are grown in a modified Lin and Staba medium supplemented with 0.2 mg/L 2,4-D, pH 5.7. Our kurodagosun 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) KNO_3

B) NH_4Cl

C) $CaCl_2 \cdot 2H_2O$

D) $MgSO_4 \cdot 7H_2O$

E) Sucrose

F) Lin_and_Staba_1

H_3BO_3	2.4 mg/mL
$MnSO_4 \cdot 5H_2O$	10.18 mg/mL
$ZnSO_4 \cdot 7H_2O$	4.05 mg/mL

G) JPL_C

$FeSO_4 \cdot 7H_2O$	2.78 mg/mL
Na_2-EDTA	3.73 mg/mL

Heat at 80℃ for 3–4 hours for chelating Fe

H) Lin_and_Staba_3

KI	0.375 mg/mL
$Na_2MoO_4 \cdot 2H_2O$	0.127 mg/mL
$CuSO_4 \cdot 5H_2O$	0.01 mg/mL
$CoCl_2 \cdot 6H_2O$	0.01 mg/mL

I) Lin_and_Staba_4

Nicotinic acid	5 mg/mL
Pyridoxine·HCl	0.5 mg/mL
Thiamine·HCl	3 mg/mL

J) Lin_and_Staba_5

KH_2PO_4	68 mg/mL

K) 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)

L) 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 modified Lin and Staba medium (medium no. 67)

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

KNO_3	5.56 g
NH_4Cl	0.268 g
$CaCl_2·2H_2O$	0.22 g
$MgSO_4·7H_2O$	0.185 g
Sucrose	20 g

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

Lin_and_Staba_1	1 mL
JPL_C	1 mL
Lin_and_Staba_3	1 mL
Lin_and_Staba_4	1 mL
Lin_and_Staba_5	1 mL
2,4-D (0.2 mg/mL)	1 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℃ for 20 min.

Methods

1. Agitate a 7-day-old culture well and transfer 10 mL of cell suspension to 80 mL of fresh modified Lin and Staba 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 kurodagosun cell suspension in a 50-mL disposable conical centrifuge tube. The cells should be transferred to fresh modified Lin and Staba medium immediately after arrival.

- For overseas customers: We send kurodagosun cells placed on semi-solid modified Lin and Staba medium in a 250-mL disposable Erlenmeyer flask. The cells should be transferred to fresh modified Lin and Staba 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 kurodagosun cell suspension cultures stably, it is essential to transfer an adequate amount of cells to fresh modified Lin and Staba medium in every subculture. The amount of cells may vary from one lab to another, because proliferation of kurodagosun 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

Fujimura T, Komamine A (1979) Synchronization of somatic embryogenesis in a carrot cell suspension culture. Plant Physiology 64: 162–164. DOI: 10.1104/pp.64.1.162

Appendix A: Formulation of culture medium

Table A.1. modified Lin and Staba medium
(medium no. 67)

Chemical	Concentration (mg/L)
KNO_3	5560
NH_4Cl	268
$CaCl_2 \cdot 2H_2O$	220
$MgSO_4 \cdot 7H_2O$	185
KH_2PO_4	68
H_3BO_3	2.4
$MnSO_4 \cdot 5H_2O$	10.18
$ZnSO_4 \cdot 7H_2O$	4.05
KI	0.375
$Na_2MoO_4 \cdot 2H_2O$	0.127
$CuSO_4 \cdot 5H_2O$	0.01
$CoCl_2 \cdot 6H_2O$	0.01
$FeSO_4 \cdot 7H_2O$	2.78
Na_2-EDTA	3.73
Nicotinic acid	5
Pyridoxine·HCl	0.5
Thiamine·HCl	3
Sucrose	20000
2,4-D sodium monohydrate	0.236