rpc00069

Coptis japonica 156-S transgenic 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: LS medium, 10 μM NAA, 0.01 μM BAP, pH 5.7 (medium no. 54) [Materials III]
- Culture conditions: 25℃, dark, 90 rpm [Methods II]
- Subculture: 21-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: "*Coptis japonica* 156-S cell line (rpc00069) was provided by the RIKEN BRC through the National BioResource Project of the MEXT, Japan."

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Introduction

Coptis japonica 156-S cell line is a transgenic cell line over-expressing *Coptis* (*S*)-scoulerine 9-*O*-methyltransferase (Sato *et al.* 2001). The 156-S is a yellow high berberine-producing cell line. The parent cell line 156-1 was established from a petiole-derived root culture of *Coptis japonica* (Thunb.) Makino var. *dissecta* (Yatabe) Nakai ex Satake (Yamada and Sato 1981, Sato and Yamada 1984). The 156-S cells are grown in a Linsmaier and Skoog (LS) medium supplemented with 10 μM 1-naphthaleneacetic acid (NAA) and 0.01 μM 6-benzylaminopurine (BAP), pH 5.7. Our 156-S cell culture has been maintained in the dark at 25℃ with rotary shaking at 90 rpm and subcultured at 21-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) LS_VT

Thiamine·HCl	0.16 mg/mL
myo-Inositol	40 mg/mL

D) NAA (1 mM)

NAA·K 0.224 mg/mL
Potassium 1-naphthylacetate, FUJIFILM Wako Pure Chemical Corporation (#161-04021)

E) BAP (1 mM)

6-Benzylaminopurine 0.225 mg/mL
Dissolve 6-benzylaminopurine in small volume of KOH (1 N), and fill up with distilled water

F) KOH (1 N)

Glassware

A) Erlenmeyer flask (300 mL), capped with two layers of aluminum foil, sterilized by autoclaving at 121℃ for 20 min

Preparation of LS medium (medium no. 54)

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.

LS_VT	2.5 mL
NAA (1 mM)	10 mL
BAP (1 mM)	0.01 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. Autoclave the medium at 121℃ for 20 min.

Methods

1. Leave to stand a flask containing a 21-day-old culture until the cells settle to the bottom of the flask.

2. Discard culture medium by decantation.

3. Wash the cells with a fresh LS medium two times. If the cell amount is too much, discard some cells with the medium.

4. Add 1.5 times the volume of fresh LS medium to the cells (up to a total volume of approximately 100 mL).

5. Incubate cell cultures on a rotary shaker at 90 rpm under the dark condition at 25℃.

Notes

- For domestic customers: We send 156-S cell suspension in a 50-mL disposable conical centrifuge tube. The cells should be transferred to fresh LS medium immediately after arrival.

- For overseas customers: We send 156-S cells placed on semi-solid LS medium in a 250-mL disposable Erlenmeyer flask. The cells should be transferred to fresh LS 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 156-S cell suspension cultures stably, it is essential to transfer an adequate amount of cells to fresh LS medium in every subculture. The amount of cells may vary from one lab to another, because proliferation of 156-S 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).

- Growth of 156-S is very slow. Do not significantly reduce the cell amount in the liquid medium.

References

Sato F, Hashimoto T, Hachiya A, Tamura K, Choi K-B, Morishige T, Fujimoto H, Yamada Y (2001) Metabolic engineering of plant alkaloid biosynthesis. Proceedings of National Academy of Sciences of USA 98: 367–372. DOI: 10.1073/pnas.98.1.367

Sato F, Yamada Y (1984) High berberine-producing cultures of *Coptis japonica* cells. Phytochemistry 23: 281–285. DOI: 10.1016/S0031-9422(00)80318-0

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Appendix A: Formulation of culture medium

Table A.1. Linsmaier and Skoog medium
(medium no. 54)

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
Thiamine·HCl	0.4
myo-Inositol	100
Sucrose	30000
NAA·K	2.24
6-Benzylaminopurine	0.00225