rpc00107

Catharanthus roseus CrB 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: mMS medium, 4.5 μM 2,4-D, pH 6.2 (medium no. 65) [Materials III]
- Culture conditions: 27℃, dark, 128 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: "*Catharanthus roseus* CrB cell line (rpc00107) was provided by the RIKEN BRC through the National BioResource Project of the MEXT, Japan."

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Introduction

Periwinkle CrB cell line was established from *Catharanthus roseus* (L.) G.Don (Takeuchi and Komamine 1978). The CrB cells are grown in a modified Murashige and Skoog (mMS) medium supplemented with 4.5 μM 2,4-dichlorophenoxyacetic acid (2,4-D), pH 6.2. Our CrB cell culture has been maintained in the dark at 27℃ with rotary shaking at 128 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) AA_VT

Nicotinic acid	0.05 mg/mL
Pyridoxine·HCl	0.05 mg/mL
Thiamine·HCl	0.01 mg/mL
Glycine	0.16 mg/mL

Store at −20℃

D) 2,4-D (1 mM)

2,4-D sodium monohydrate 0.261 mg/mL
(2,4-Dichlorophenoxy)acetic acid sodium salt monohydrate, Sigma-Aldrich (D6679)

E) NaOH (1 N)

Glassware

A) Erlenmeyer flask (100 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 mMS medium (medium no. 65)

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.

AA_VT	10 mL
2,4-D (1 mM)	4.5 mL

3. Adjust the pH of the solution to 6.2 with NaOH (1 N), and fill up to 1 L with distilled water.

4. Pour 20 mL of the medium into a 100-mL flask.

5. Autoclave the flask at 121℃ for 20 min.

Methods

1. Agitate a 7-day-old culture well and transfer 2.5 mL of cell suspension to 20 mL of fresh mMS medium with a pipette.

2. Incubate cell cultures on a rotary shaker at 128 rpm under the dark condition at 27℃.

Notes

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

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

Takeuchi Y, Komamine A (1978) Changes in the composition of cell wall polysaccharides of suspension-cultured *Vinca rosea* cells during culture. Physiologia Plantarum, 42: 21–28. DOI: 10.1111/j.1399-3054.1978.tb01532.x

Appendix A: Formulation of culture medium

Table A.1. modified Murashige and Skoog medium (medium no. 65)

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	1.6
Sucrose	30000
2,4-D sodium monohydrate	1.18