rpc00049

Vitis vinifera YU-1 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: mB5 medium, 0.54 mg/L NAA, 0.2 mg/L kinetin, pH 5.7 (medium no. 41) [Materials III]
- Culture conditions: 27°C, dark, 100 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: "*Vitis vinifera* YU-1 cell line (rpc00049) was provided by the RIKEN BRC through the National BioResource Project of the MEXT, Japan."

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Introduction

Grape YU-1 cell line was established from a shoot apex of *Vitis vinifera* L. cultivar Koshu (Fujita *et al*. 2018). The YU-1 cells are grown in a modified Gamborg's B5 (mB5) medium supplemented with 0.54 mg/L 1-naphthaleneacetic acid (NAA) and 0.2 mg/L kinetin, pH 5.7. Our YU-1 cell culture has been maintained in the dark at 27℃ with rotary shaking at 100 rpm and subcultured at 7-day intervals.

Materials

Chemicals and stock solutions

(All stock solutions are stored at 4℃)

A) B5 salt mix

Gamborg's B5 Medium Salt Mixture, FUJIFILM Wako Pure Chemical Corporation (#399-00621)

B) Sucrose

C) B5_VT

Nicotinic acid	0.4 mg/mL
Pyridoxine·HCl	0.4 mg/mL
Thiamine·HCl	4 mg/mL
myo-Inositol	40 mg/mL

D) NAA (1 mg/mL)

NAA·K 1.2 mg/mL
Potassium 1-naphthylacetate, FUJIFILM Wako Pure Chemical Corporation (#161-04021)

E) Kinetin (0.2 mg/mL)

Kinetin 0.2 mg/mL
Dissolve kinetin in small volume of KOH (1 N), and fill up with distilled water

F) KOH (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 mB5 medium (medium no. 41)

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

B5 salt mix	1 bag (1 L)
Sucrose	19.85 g

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

B5_VT	5 mL
NAA (1 mg/mL)	0.54 mL
Kinetin (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 30 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 2.6–4 mL of cell suspension to 30 mL of fresh mB5 medium with a pipette.

2. Incubate cell cultures on a rotary shaker at 100 rpm under the dark condition at 27℃.

Notes

- For domestic customers: We send YU-1 cell suspension in a 50-mL disposable conical centrifuge tube. The cells should be transferred to fresh mB5 medium immediately after arrival.

- For overseas customers: We send YU-1 cells placed on semi-solid mB5 medium in a 250-mL disposable Erlenmeyer flask. The cells should be transferred to fresh mB5 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 YU-1 cell suspension cultures stably, it is essential to transfer an adequate amount of cells to fresh mB5 medium in every subculture. The amount of cells may vary from one lab to another, because proliferation of YU-1 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

Fujita K, Aoki Y, Suzuki S (2018) Antidiabetic effects of novel cell culture established from grapevine, *Vitis vinifera* cv. Koshu. Cytotechnology 70: 993–999. DOI: 10.1007/s10616-018-0203-y

Appendix A: Formulation of culture medium

Table A.1. modified Gamborg's B5 medium
(medium no. 41)

Chemical	Concentration (mg/L)
KNO_3	2500
$(NH_4)2SO_4$	134
$CaCl_2 \cdot 2H_2O$	150
$MgSO_4 \cdot 7H_2O$	250
$NaH_2PO_4 \cdot H_2O$	150
H_3BO_3	3
$MnSO_4 \cdot H_2O$	10
$ZnSO_4 \cdot 7H_2O$	2
KI	0.75
$Na_2MoO_4 \cdot 2H_2O$	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	2
Pyridoxine·HCl	2
Thiamine·HCl	20
myo-Inositol	200
Sucrose	19850
NAA·K	0.648
Kinetin	0.2