rpc00016

Spinacia oleracea Spi-WT 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, 0.5 mg/L NAA, 1 mg/L BAP, pH 6.5 (medium no. 12) [Materials III]
- Culture conditions: 27°C, dark, 140 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: "*Spinacia oleracea* Spi-WT cell line (rpc00016) was provided by the RIKEN BRC through the National BioResource Project of the MEXT, Japan."

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Introduction

Spinach Spi-WT cell line was established from *Spinacia oleracea* L. (Nakagawa *et al*. 1985). The Spi-WT cells are grown in a modified Murashige and Skoog (mMS) medium supplemented with 0.5 mg/L 1-naphthaleneacetic acid (NAA) and 1 mg/L 6-benzylaminopurine (BAP), pH 6.5. Our Spi-WT cell culture has been maintained in the dark at 27°C with rotary shaking at 140 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

myo-Inositol	40 mg/mL

E) NAA (1 mg/mL)

NAA·K	1.2 mg/mL

Potassium 1-naphthylacetate, FUJIFILM Wako Pure Chemical Corporation (#161–04021)

F) BAP (1 mg/mL)

6-Benzylaminopurine	1 mg/mL

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

G) NaOH (1 N)

Glassware and equipment

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

C) Plastic conical centrifuge tube (50 mL), sterile

Preparation of mMS medium (medium no. 12)

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

MS salt mix	1 bag (1 L)
Sucrose	20 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
NAA (1 mg/mL)	0.5 mL
BAP (1 mg/mL)	1 mL

3. Adjust the pH of the solution to 6.5 with NaOH (1 N), and fill up to 1 L with distilled water.

4. Pour 25 mL of the medium into a 100-mL flask.

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

Methods

1. Centrifuge 7-day-old culture in a 50-mL conical centrifuge tube at 100 $\times g$ for 5 min and discard the supernatant.

2. Resuspend the pelleted cells in fresh mMS medium (total volume 30 mL).

3. Transfer 15 mL of cell suspension to 25 mL of fresh mMS medium with a pipette.

4. Incubate cell cultures on a rotary shaker at 140 rpm under the dark condition at 27℃.

Notes

- For domestic customers: We send Spi-WT cell suspension in a 50-mL disposable conical centrifuge tube. The cells should be transferred to fresh mMS medium immediately after arrival. Transfer settled cells to Erlenmeyer flasks containing fresh liquid medium with a pipette.

- For overseas customers: We send Spi-WT 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 Spi-WT 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 Spi-WT 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

Nakagawa H, Tanaka H, Oba T, Ogura N, Iizuka M (1985) Callus formation from protoplasts of cultured *Spinacia oleracea* cells. Plant Cell Reports 4: 148–150. DOI: 10.1007/BF00571303

Appendix A: Formulation of culture medium

Table A1. modified Murashige and Skoog medium (medium no. 12)

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	2
myo-Inositol	100
Sucrose	20000
NAA·K	0.6
6-Benzylaminopurine	1