

rpc00018

Spinacia oleracea* Spi-12F 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. 13) [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-12F cell line (rpc00018) was provided by the RIKEN BRC through the National BioResource Project of the MEXT, Japan.”

Experimental Plant Division
RIKEN BioResource Research Center (BRC)
Koyadai 3-1-1, Tsukuba, Ibaraki 305-0074
Japan
FAX: +81 29 836 9053
E-mail: plant.brc@riken.jp
<http://epd.brc.riken.jp/en/>

Introduction

Spinach Spi-12F cell line was established from *Spinacia oleracea* L. (Nakagawa *et al.* 1985). The Spi-12F 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-benzylamino-purine (BAP), pH 6.5. Our Spi-12F 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) KCl
- B) L-Glutamine
- C) NZ-Amine Type A
- D) CaCl₂·2H₂O
- E) MgSO₄·7H₂O
- F) KH₂PO₄
- G) Sucrose
- H) MS_micro_1

H ₃ BO ₃	0.62 mg/mL
MnSO ₄ ·5H ₂ O	2.41 mg/mL
ZnSO ₄ ·7H ₂ O	0.86 mg/mL
KI	0.083 mg/mL
Na ₂ MoO ₄ ·2H ₂ O	0.025 mg/mL
CuSO ₄ ·5H ₂ O	0.0025 mg/mL
CoCl ₂ ·6H ₂ O	0.0025 mg/mL

- I) MS_micro_2

FeSO ₄ ·7H ₂ O	2.78 mg/mL
Na ₂ -EDTA	3.73 mg/mL
Heat at 80°C for 3–4 hours for chelating Fe	

- J) MS_VT

Nicotinic acid	0.5 mg/mL
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Pyridoxine·HCl	0.5 mg/mL
Thiamine·HCl	0.1 mg/mL
Glycine	2 mg/mL

K) MS_inositol

<i>myo</i> -Inositol	40 mg/mL
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L) NAA (1 mg/mL)

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

M) 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	

N) 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°C for 20 min
- C) Plastic conical centrifuge tube (50 mL), sterile

Preparation of mMS medium (medium no. 13)

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

KCl	1.4 g
L-Glutamine	1 g
NZ-Amine Type A	1 g
CaCl ₂ ·2H ₂ O	0.44 g
MgSO ₄ ·7H ₂ O	0.37 g
KH ₂ PO ₄	0.17 g
Sucrose	20 g

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

MS_micro_1	10 mL
MS_micro_2	10 mL

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°C for 20 min.

Methods

1. Centrifuge 7-day-old culture in a 50-mL conical centrifuge tube at 100 ×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°C.

Notes

- For domestic customers: We send Spi-12F 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-12F 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-12F 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-12F 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 Poly-

mer, 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](https://doi.org/10.1007/BF00571303)

Appendix A: Formulation of culture medium

Table A1. modified Murashige and Skoog medium
(medium no. 13)

Chemical	Concentration (mg/L)
KCl	1400
L-Glutamine	1000
NZ-Amine Type A	1000
CaCl ₂ ·2H ₂ O	440
MgSO ₄ ·7H ₂ O	370
KH ₂ PO ₄	170
H ₃ BO ₃	6.2
MnSO ₄ ·5H ₂ O	24.1
ZnSO ₄ ·7H ₂ O	8.6
KI	0.83
Na ₂ MoO ₄ ·2H ₂ O	0.25
CuSO ₄ ·5H ₂ O	0.025
CoCl ₂ ·6H ₂ O	0.025
FeSO ₄ ·7H ₂ O	27.8
Na ₂ -EDTA	37.3
Nicotinic acid	0.5
Pyridoxine·HCl	0.5
Thiamine·HCl	0.1
Glycine	2
<i>myo</i> -Inositol	100
Sucrose	20000
NAA·K	0.6
6-Benzylaminopurine	1