rpc00060

# *Glycyrrhiza echinata* Ge callus culture

**Components**

- A 9-cm plastic Petri dish, containing cells placed on semi-solid medium

**Notice**

- Subculture the cells to fresh medium immediately after arrival [Notes I].
- 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: MS medium, 1 mg/L IAA, 0.1 mg/L kinetin, 0.9% (w/v) agar, pH 5.8 (medium no. 49) [Materials III]
- Culture conditions: 25–27°C, dark [Methods II]
- Subculture: 28-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: "*Glycyrrhiza echinata* Ge cell line (rpc00060) was provided by the RIKEN BRC through the National BioResource Project of the MEXT, Japan."

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## Introduction

Licorice Ge cell line was established from *Glycyrrhiza echinata* L. (Nakamura *et al*. 1999). The Ge callus culture has the capacity for producing a yellow pigment, a retrochalcone, by elicitor treatment. The Ge cells are grown on a Murashige and Skoog (MS) medium supplemented with 1 mg/L indole-3-acetic acid (IAA) and 0.1 mg/L kinetin, and solidified with 0.9% (w/v) agar, pH 5.8. Our Ge cell culture has been maintained in the dark at 25–27°C and subcultured at 28-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) IAA (1 mg/mL)

| | |
|---|---|
| IAA·K | 1.217 mg/mL |

Potassium 3-indoleacetate, FUJIFILM Wako Pure Chemical Corporation (#160-07531)

F) 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

G) Agar, powder

H) KOH (1 N)

### Glassware and equipment

A) Erlenmeyer flask (100 mL), capped with two layers of aluminum foil

B) Forceps, sterilized before use

### Preparation of MS medium (medium no. 49)

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.

| | |
|---|---|
| MS_VT | 1 mL |
| MS_inositol | 2.5 mL |
| IAA (1 mg/mL) | 1 mL |
| Kinetin (0.2 mg/mL) | 0.5 mL |

3. Adjust the pH of the solution to 5.8 with KOH (1 N), and fill up to 1 L with distilled water.

4. Pour 40 mL of the medium into a 100-mL flask containing 0.36 g of agar.

5. Autoclave the flask at 121°C for 20 min.

## Methods

1. Pick up an appropriate amount of callus cells from a 28-day-old culture with a forceps and place the cells onto fresh MS medium.

2. Incubate cell cultures under the dark condition at 25–27°C.

## Notes

- We send Ge cells on semi-solid MS medium in a 9-cm disposable Petri dish. The cells should be subcultured to fresh MS medium immediately after arrival.

- In order to maintain Ge callus culture stably, it is essential to observe the growth of cells carefully. Because proliferation of Ge cells is affected by culture conditions, such as a room temperature, aeration conditions of the culture and so on, an amount of cells transferred to fresh medium and the subculture intervals may vary from one lab to another. We usually inoculate three to four pieces of Ge callus (about 3–5-mm

in diameter) on 40 mL of MS medium in a 100-mL flask, and culture them for 28 days.

## References

Nakamura K, Akashi T, Aoki T, Kawaguchi K, Ayabe S (1999) Induction of isoflavonoid and retrochalcone branches of the flavonoid pathway in cultured *Glycyrrhiza echinata* cells treated with yeast extract. Bioscience, Biotechnology, and Biochemistry 63: 1618–1620. DOI: 10.1271/bbb.63.1618

## Appendix A: Formulation of culture medium

Table A.1. Murashige and Skoog medium
(medium no. 49)

| 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 | 30000 |
| IAA·K | 1.217 |
| Kinetin | 0.1 |
| Agar | 9000 |