rpc00100

# *Athyrium yokoscense* AY-01 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: 1/2mMS medium, 1 mg/L kinetin, pH 5.8 (medium no. 60) [Materials III]
- Culture conditions: 24℃, continuous light, 100 rpm [Methods II]
- Subculture: 14-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: "*Athyrium yokoscense* AY-01 cell line (rpc00100) 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

AY-01 cell line was established from a leaf of a metal hyper-tolerant fern *Athyrium yokoscense* (Franch. & Sav.) Christ (Yoshihara *et al.* 2005). The AY-01 cells are grown in a 1/2 modified Murashige and Skoog (1/2mMS) medium supplemented with 1 mg/L kinetin, pH 5.8. Our AY-01 cell culture has been maintained under the continuous light at 24℃ with rotary shaking at 100 rpm and subcultured at 14-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) 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) 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 and equipment

A) Erlenmeyer flask (300 mL), capped with two layers of aluminum foil

B) Forceps, sterilized before use

### Preparation of 1/2mMS medium (medium no. 60)

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

| | |
|---|---|
| MS salt mix | 0.5 bag (equivalent with 0.5 L) |
| Sucrose | 30 g |

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

| | |
|---|---|
| MS_VT | 0.5 mL |
| MS_inositol | 1.25 mL |
| Kinetin (0.2 mg/mL) | 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 100 mL of the medium into a 300-mL flask.

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

## Methods

1. Transfer seven to nine cell clumps from 14-day-old culture to 100 mL of fresh 1/2mMS medium with a forceps.

2. Incubate cell cultures on a rotary shaker at 100 rpm under the continuous light condition (photosynthetic photon flux density 60 μmol $m^{-2}$ $s^{-1}$) at 24℃.

## Notes

- For domestic customers: We send AY-01 cell suspension in a 50-mL disposable conical centrifuge tube. The cells should be transferred to fresh 1/2mMS medium immediately after arrival.

- For overseas customers: We send AY-01 cells placed on semi-solid 1/2mMS medium in a 250-mL disposable Erlenmeyer flask. The cells should be transferred to fresh 1/2mMS medium immediately after arrival. Transfer the cell clumps to Erlenmeyer flasks containing fresh liquid medium with a forceps.

- In order to maintain AY-01 cell suspension cultures stably, it is essential to transfer an adequate amount of cells to fresh 1/2mMS medium in every subculture. The amount of cells may vary from one lab to another, because proliferation of AY-01 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

Yoshihara T, Tsunokawa K, Miyano Y, Arashima Y, Hodoshima H, Shoji K, Shimada H, Goto F (2005) Induction of callus from a metal hypertolerant fern, *Athyrium yokoscense*, and evaluation of its cadmium tolerance and accumulation capacity. Plant Cell Reports 23: 579–585. DOI: 10.1007/s00299-004-0877-9

## Appendix A: Formulation of culture medium

Table A.1. 1/2 modified Murashige and Skoog medium (medium no. 60)

| Chemical | Concentration (mg/L) |
|---|---|
| $KNO_3$ | 950 |
| $NH_4NO_3$ | 825 |
| $CaCl_2 \cdot 2H_2O$ | 220 |
| $MgSO_4 \cdot 7H_2O$ | 185 |
| $KH_2PO_4$ | 85 |
| $H_3BO_3$ | 3.1 |
| $MnSO_4 \cdot 4H_2O$ | 11.15 |
| $ZnSO_4 \cdot 7H_2O$ | 4.3 |
| KI | 0.415 |
| $Na_2MoO_4 \cdot 2H_2O$ | 0.125 |
| $CuSO_4 \cdot 5H_2O$ | 0.0125 |
| $CoCl_2 \cdot 6H_2O$ | 0.0125 |
| $FeSO_4 \cdot 7H_2O$ | 13.9 |
| $Na_2$-EDTA | 18.65 |
| Nicotinic acid | 0.25 |
| Pyridoxine·HCl | 0.25 |
| Thiamine·HCl | 0.05 |
| Glycine | 1 |
| *myo*-Inositol | 50 |
| Sucrose | 30000 |
| Kinetin | 1 |