

rpc00065

***Amaranthus* H440 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, 0.25% (w/v) gellan gum, pH 5.7 (medium no. 44) [[Materials III](#)]
- Culture conditions: 25°C, continuous light [[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: “*Amaranthus* H440 cell line (rpc00065) was provided by the RIKEN BRC through the National BioResource Project of the MEXT, Japan.”

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## Introduction

Amaranth H440 cell line was established from a leaf of *Amaranthus* sp. (Asano 2011, Asano and Otake 2011). The H440 callus cells are dark red and produce betacyanin. The H440 cells are grown on a phytohormone-free Murashige and Skoog (MS) medium solidified with 0.25% (w/v) gellan gum, pH 5.7. Our H440 cell culture has been maintained under the continuous light at 25°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

<i>myo</i> -Inositol	40 mg/mL
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#### E) Gellan gum

Gellan gum, FUJIFILM Wako Pure Chemical Corporation (#073-03071)

#### F) KOH (1 N)

### Glassware and equipment

#### A) Erlenmeyer flask (200 mL), capped with two layers of aluminum foil

#### B) Forceps, sterilized before use

### Preparation of MS medium (medium no. 44)

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

MS salt mix	1 bag (1 L)
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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

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 60 mL of the medium into a 200-mL flask containing 0.15 g of gellan gum.
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 continuous light condition (photosynthetic photon flux density 45–50  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) at 25°C.

## Notes

- We send H440 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 H440 callus culture stably, it is essential to observe the growth of cells carefully. Because proliferation of H440 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 five pieces of H440 callus (about 3–10-mm in diameter) on 60 mL of MS medium in a 200-mL flask, and culture them for 28 days.
- Bright pink H440 cells are suitable for subculture.

## References

Asano S (2011) Research into pigment production using cultured plant cells grown without phytohormones. PhD thesis, University of Tsukuba, Japan (in Japanese). <http://hdl.handle.net/2241/114680>

Asano S, Otake K (2011) Production of phytochemicals by using habituated and long-term cultured cells. *Plant Biotechnology* 28: 51–62. DOI: [10.5511/plantbiotechnology.10.1109a](https://doi.org/10.5511/plantbiotechnology.10.1109a)

## Appendix A: Formulation of culture medium

Table A.1. Murashige and Skoog medium  
(medium no. 44)

Chemical	Concentration (mg/L)
KNO <sub>3</sub>	1900
NH <sub>4</sub> NO <sub>3</sub>	1650
CaCl <sub>2</sub> ·2H <sub>2</sub> O	440
MgSO <sub>4</sub> ·7H <sub>2</sub> O	370
KH <sub>2</sub> PO <sub>4</sub>	170
H <sub>3</sub> BO <sub>3</sub>	6.2
MnSO <sub>4</sub> ·4H <sub>2</sub> O	22.3
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	8.6
KI	0.83
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	0.25
CuSO <sub>4</sub> ·5H <sub>2</sub> O	0.025
CoCl <sub>2</sub> ·6H <sub>2</sub> O	0.025
FeSO <sub>4</sub> ·7H <sub>2</sub> O	27.8
Na <sub>2</sub> -EDTA	37.3
Nicotinic acid	0.5
Pyridoxine·HCl	0.5
Thiamine·HCl	0.1
Glycine	2
<i>myo</i> -Inositol	100
Sucrose	30000
Gellan gum	2500