rpc00001

**Nicotiana tabacum** BY-2 cell suspension culture

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**Components**

- Domestic delivery: Two 50-mL tubes, containing 25 mL of cell suspension
- Overseas delivery: Two 250-mL flasks, 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.

**Summary**

- Culture medium: mLS medium, 0.2 mg/L 2,4-D, pH 5.8 (medium no. 1) [Materials III]
- Culture conditions: 27°C, dark, 130 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: “*Nicotiana tabacum* BY-2 cell line (rpc00001) was provided by the RIKEN BRC which is participating in the National BioResource Project of the MEXT/AMED, Japan.”

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Introduction

Tobacco BY-2 is a fast-growing and highly-homogeneous cell line, and used for a broad range of plant studies around the world (Nagata et al. 2006). A suspension culture was established from a callus induced from a seedling of *Nicotiana tabacum* L. cultivar Bright Yellow 2 (Nagata et al. 1992). The BY-2 cells are grown in a modified Linsmaier and Skoog (mLS) medium supplemented with 0.2 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D), pH 5.8. Our BY-2 cell culture has been maintained in the dark at 27°C with rotary shaking at 130 rpm and subcultured at 7-day intervals (Figure 1, 2).

Materials

I. Chemicals and stock solutions
   (All stock solutions are stored at 4°C)

   A) MS salt mix
      Murashige and Skoog Plant Salt Mixture, Wako Pure Chemical Industries (#392-00591)

   B) Sucrose

   C) BY2_P
      \[ \text{KH}_2\text{PO}_4 \] 80 mg/mL

   D) LS_VT_modified
      Thiamine-HCl 0.4 mg/mL
      myo-Inositol 40 mg/mL

   E) 2,4-D (0.2 mg/mL)
      2,4-D sodium monohydrate 0.236 mg/mL
      (2,4-Dichlorophenoxy)acetic acid sodium salt monohydrate, Sigma-Aldrich (D6679)

   F) KOH (1 N)

II. Glassware

   A) Erlenmeyer flask (300 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

III. Preparation of mLS medium (medium no. 1)

   A) Dissolve the following chemicals in approximately 800 mL of distilled water.
MS salt mix 1 bag (1 L)
Sucrose 30 g

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

BY2_P 2.5 mL
LS_VT_modified 2.5 mL
2,4-D (0.2 mg/mL) 1 mL

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

D) Pour 95 mL of the medium into a 300-mL flask.

E) Autoclave the flask at 121°C for 20 min.

Methods

I. Agitate a 7-day-old culture well and transfer 1–1.2 mL of cell suspension to 95 mL of fresh mLS medium with a pipette.

II. Incubate cell cultures on a rotary shaker at 130 rpm under the dark condition at 27°C.

Notes

I. For domestic customers: We send BY-2 cell suspension in 50-mL disposable tubes. The cells should be transferred to fresh mLS medium immediately after arrival. Transfer settled cells to Erlenmeyer flasks containing fresh liquid medium with a pipette.

II. For overseas customers: We send BY-2 cells placed on semi-solid mLS medium in 250-mL disposable flasks. The cells should be transferred to fresh mLS 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.

III. In order to maintain BY-2 cell suspension cultures stably, it is essential to transfer an adequate amount of cells to fresh mLS medium in every subculture. The amount of cells may vary from one lab to another, because proliferation of BY-2 cells is affected by culture conditions, such as a room temperature, rotation speed of a rotary shaker, and aeration condition of the culture.

IV. A low growth rate of BY-2 cells is sometimes caused by poor aeration (Kuma-
gai-Sano et al. 2007). In order to obtain good aeration of a suspension culture, a silicone cap may be used instead of the aluminum foil cap.

References


Figure 1: *Nicotiana tabacum* BY-2 cell suspension culture

A: Seven-day-old cell suspension culture.

B: Morphology of BY-2 cells after 3 day of subculture. BY-2 cells were observed by using differential interference contrast microscopy. Scale bar = 100 µm
Figure 2: Growth profile of BY-2 cells
Appendix A

Table A1: Formulation for modified Linsmaier and Skoog medium (medium no. 1)

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Concentration (mg/L)</th>
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<tbody>
<tr>
<td>KNO₃</td>
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<tr>
<td>NH₄NO₃</td>
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</tr>
<tr>
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<td>Sucrose</td>
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<tr>
<td>2,4-D sodium monohydrate</td>
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