rpc00036-BY-2H-3.3 (2014.10.3; revised 2024.1.29)
RIKEN BRC plant cell line documentation

rpc00036

Nicotiana tabacum BY-2H 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: mLS medium, 0.9% (w/v) agar, pH 5.8 (medium no. 31) [Materials III]
- Culture conditions: 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: "*Nicotiana tabacum* BY-2H cell line (rpc00036) 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

Tobacco BY-2H cell line is a habituated BY-2 cell line that have capacity to proliferate without 2,4-dichlorophenoxyacetic acid (2,4-D) (Syōno and Fujita 1994). The parent cell line BY-2 (rpc00001) was established from a callus induced from a seedling of *Nicotiana tabacum* L. cultivar Bright Yellow 2 in the presence of 2,4-D (Nagata *et al*. 1992). The BY-2H cells are grown on a phytohormone-free modified Linsmaier and Skoog (mLS) medium solidified with 0.9% (w/v) agar, pH 5.8. Our BY-2H cell culture has been maintained in the dark at 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) BY2_P

KH_2PO_4	80 mg/mL

D) LS_VT_modified

Thiamine·HCl	0.4 mg/mL
myo-Inositol	40 mg/mL

E) Agar, powder

F) 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 mLS medium (medium no. 31)

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.

BY2_P	2.5 mL
LS_VT_modified	2.5 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℃ 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 mLS medium.

2. Incubate cell cultures under the dark condition at 27℃.

Notes

- We send BY-2H cells on semi-solid mLS medium in a 9-cm disposable Petri dish. The cells should be subcultured to fresh mLS medium immediately after arrival.

- In order to maintain BY-2H callus culture stably, it is essential to observe the growth of cells carefully. Because proliferation of BY-2H 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 pieces of BY-2H callus (about 5–7-mm in diameter) on 40 mL of mLS medium in a 100-mL flask, and culture them for 28 days.

- It is important to subculture good healthy cells. Brownish BY-2H cells near the upper part of a callus should not be used.

References

Nagata T, Nemoto Y, Hasezawa S (1992) Tobacco BY-2 cell line as the “HeLa” cell in the cell biology of higher plants. International Review of Cytology 132: 1–30. DOI: 10.1016/S0074-7696(08)62452-3

Syōno K, Fujita T (1994) Habituation as a tumorous state that is interchangeable with a normal state in plant cells. International Review of Cytology 152: 265–299. DOI: 10.1016/S0074-7696(08)62559-0

Appendix A: Formulation of culture medium

Table A.1. modified Linsmaier and Skoog medium
(medium no. 31)

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	370
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
Thiamine·HCl	1
myo-Inositol	100
Sucrose	30000
Agar	9000