rpc00091

Nicotiana tabacum TBY2-31/ST transgenic 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: mLS medium, 0.2 mg/L 2,4-D, pH 5.8 (medium no. 1) [Materials III]
- Culture conditions: 27℃, dark, 110 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* TBY2-31/ST cell line (rpc00091) was provided by the RIKEN BRC through the National BioResource Project of the MEXT, Japan."

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Introduction

Tobacco TBY2-31/ST cell line is a transgenic BY-2 cell line expressing Green Fluorescent Protein (GFP) fused with Arabidopsis SYP31 and monomeric Red Fluorescent Protein (mRFP) fused with rat sialyltransferase (Ito *et al*. 2012). GFP fluorescence is observed in *cis*-Golgi, and mRFP fluorescence is in *trans*-Golgi, using a fluorescence microscope. The parent cell line BY-2 (rpc00001) was established from a callus induced from a seedling of *Nicotiana tabacum* L. cultivar Bright Yellow 2 (Nagata *et al*. 1992). The TBY2-31/ST 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 TBY2-31/ST cell culture has been maintained in the dark at 27℃ with rotary shaking at 110 rpm and subcultured at 7-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) BY2_P

KH_2PO_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)

Glassware

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

B) Pipette (2 mL; large tip opening) and a bulb, sterilized by autoclaving at 121℃ for 20 min

Preparation of mLS medium (medium no. 1)

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
2,4-D (0.2 mg/mL)	1 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 30 mL of the medium into a 100-mL flask.

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

Methods

1. Agitate a 7-day-old culture well and transfer 0.4 mL of cell suspension to 30 mL of fresh mLS medium with a pipette.

2. Incubate cell cultures on a rotary shaker at 110 rpm under the dark condition at 27℃.

Notes

- For domestic customers: We send TBY2-31/ST cell suspension in a 50-mL disposable conical centrifuge tube. The cells should be transferred to fresh mLS medium immediately after arrival.

- For overseas customers: We send TBY2-31/ST cells placed on semi-solid mLS medium in a 250-mL disposable Erlenmeyer flask. 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.

- In order to maintain TBY2-31/ST 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 TBY2-31/ST cells is affected by culture conditions, such as a room temperature, rotation speed of a rotary shaker, and aeration condition of the culture.

- A low growth rate of the parent BY-2 cells is sometimes caused by poor aeration (Kumagai-Sano *et al*. 2007). 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

Kumagai-Sano F, Hayashi T, Sano T, Hasezawa S (2007) Cell cycle synchronization of tobacco BY-2 cells. Nature Protocols 1: 2621–2627. DOI: 10.1038/nprot.2006.381

Ito Y, Uemura T, Shoda K, Fujimoto M, Ueda T, Nakano A (2012) *cis*-Golgi proteins accumulate near the ER exit sites and act as the scaffold for Golgi regeneration after brefeldin A treatment in tobacco BY-2 cells. Molecular Biology of the Cell 23: 3203–3214. DOI: 10.1091/mbc.E12-01-0034

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

Appendix A: Formulation of culture medium

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

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
2,4-D sodium monohydrate	0.236