SPiDER-βGal

Technical Manual

Technical Manual (Japanese version) is available at http://www.dojindo.co.jp/manual/sg02.pdf

General Information

The gene of β-galactosidase from *E. coli* is widely used as a reporter gene assay marker. Although X-gal is well known reagent to detect β-galactosidase in cell or tissue samples, the assay using these reagents require to fix cells or tissues due to the poor cell-permeability. In addition, so far developed the assay using fluorescence reagents can not clearly differentiate β-galactosidase-expressed cells or regions.

To overcome these issues, Urano, Kamiya and co-workers have successfully developed SPiDER-βGal. SPiDER-βGal ideally possesses cell-permeability and the ability to retain in intracellular region.[1)]

By the enzymatic reaction, SPiDER-βGal immediately forms a quinone methide that acts as electrophile when proteins containing nucleophilic functional groups nearby the molecules. By the probe undergoes the reaction with a protein, the conjugates become fluorescent compounds. Thus, SPiDER-βGal allows a single-cell analysis because it does self-immobilizing to the intracellular proteins.

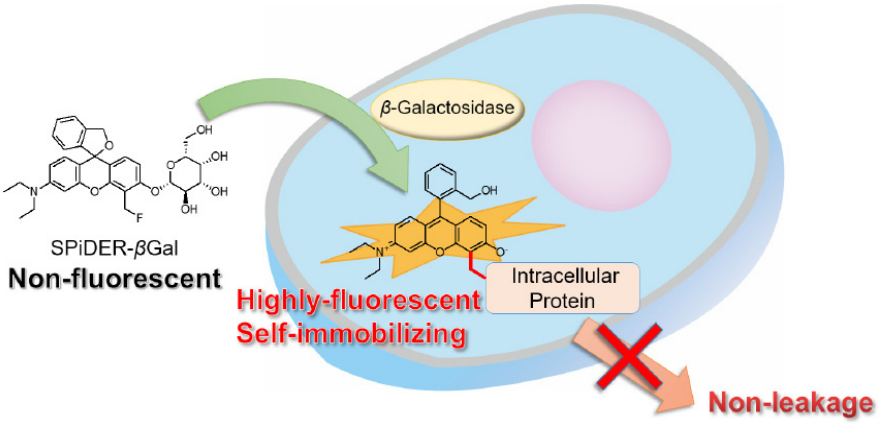

Fig. 1 Cell staining mechanism by SPiDER-βGal.

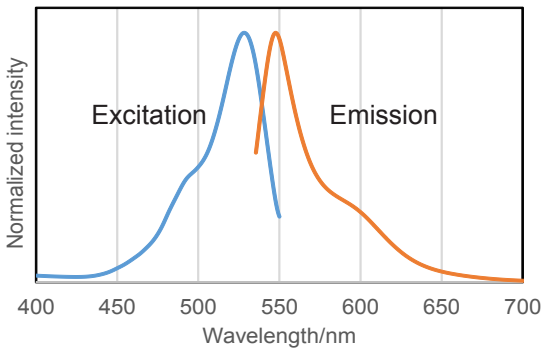

Fig. 2 Excitation and emission spectra of SPiDER-βGal after reaction with β-galactosidase.

Contents

- SPiDER-βGal 20 µg x 3

Storage Condition

Store at 0-5 °C

Required Equipment and Materials

- Dimethyl sulfoxide (DMSO)
- Hanks' HEPES buffer
- Micropipettes
- Microtubes

Preparation of solutions

Preparation of 1 mmol/l SPiDER-βGal DMSO stock solution

Add 35 µl of DMSO to a tube of SPiDER-βGal (20 µg) and dissolve it with pipetting.

*Store the SPiDER-βGal stock solution at -20°C.

Preparation of 1 µmol/l SPiDER-βGal working solution

Dilute the SPiDER-βGal DMSO stock solution with Hanks' HEPES buffer to prepare 1 µmol/l SPiDER-βGal working solution.

*Hanks' HEPES buffer is recommended to maintain cell condition.

General protocol

SPiDER-βGal staining

1. Prepare cells for the assay.
2. Discard the culture medium and wash the cells with Hanks' HEPES buffer twice.
3. Add an appropriate volume of SPiDER-βGal working solution.
4. Incubate at 37°C for 15 minutes.
5. Observe the cells under a fluorescence microscope or by a flow cytometer.

*After staining, the cells can be observed even without washing. However, you can wash it as needed.

Usage examples

Fluorescence microscopic detection of β-galactosidase-expressed cells

1. HEK cells at 5 × 10^5 cells/ml (500 µl) and HEK/LacZ cells at 5 × 10^5 cells/ml (500 µl) were seeded in a 35 mm dish in DMEM (10% fetal bovine serum, 1% penicillin-streptmycin) and cultured overnight in a 5% CO_2 incubator at 37°C.
2. The cells were washed with 2 ml of Hanks' HEPES buffer twice.
3. SPiDER-βGal working solution (2 ml) was added to the culture dish, and the cells were incubated for 15 minutes at 37°C.
4. After the supernatant was removed, the cells were washed Hanks' HEPES buffer (2 ml) twice.
5. Hanks' HEPES buffer (2 ml) were added, and the cells were observed under a fluorescence microscope. (Fig. 3A)
6. After the supernatant was removed, 4% paraformaldehyde (PFA) /PBS solution (2 ml) was added to the culture dish, and the cells were incubated for 15 minutes at room temperature.
7. After 4% PFA/PBS solution was removed, the cells were washed Hanks' HEPES buffer (2 ml) twice.
8. Hanks' HEPES buffer (2 ml) were added, and the cells were observed under a fluorescence microscope. (Fig. 3B)

*Filter (wavelength/band pass)
Fluorescence imaging: 550/25 nm (Ex), 605/70 nm (Em)

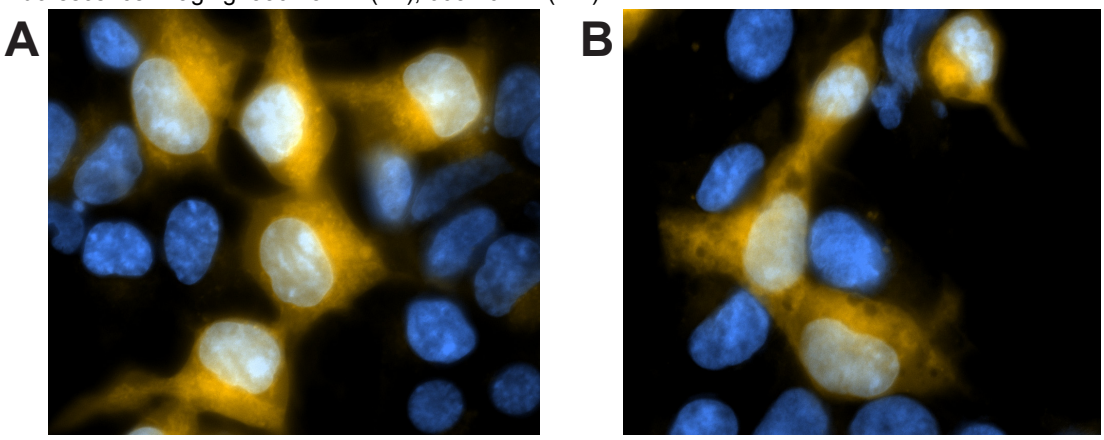

Fig. 3 Fluorescence imaging of HEK/LacZ cells and HEK cells at 1: 1 ratio.
A. living cell, B. fixing cells (4% PFA/PBS)
(yellow: SPiDER-βGal, blue: Hoechst 33342)

β-galactosidase-expressed cells (HEK/LacZ cells) were clearly observed in fluorescence imaging. In addition, the result was not changed by fixing the cells.

Flow cytometric detection of β-galactosidase-expressed cells

1. HEK cells at 5 × 10^5 cells/ml (500 µl) and HEK/LacZ cells at 5 × 10^5 cells/ml (500 µl) were mixed in a microtube.
2. SPiDER-βGal DMSO stock solution (1 µl) was added to the tube, and the cells were incubated for 15 minutes at 37°C .
3. The cells were analyzed by a flow cytometer. (488 nm excitation, 530/30 nm bandpass filter)

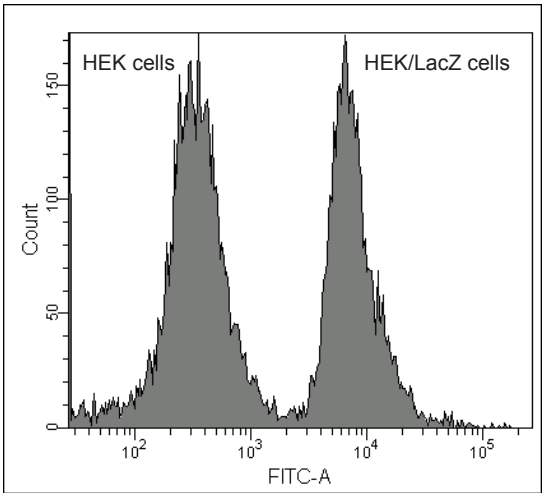

Fig. 4 Analysis of HEK/LacZ cells and HEK cells at 1: 1 ratio by flow cytometry.

β-galactosidase-expressed cells (HEK/LacZ cells) were clearly differentiate from HEK cells in flow cytometry data analysis.

Reference

1) Y. Urano, M. Kamiya, T. Doura, WO 2015174460, A1, (19, November, 2015).

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If you need more information, please contact Dojindo technical service.

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