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.

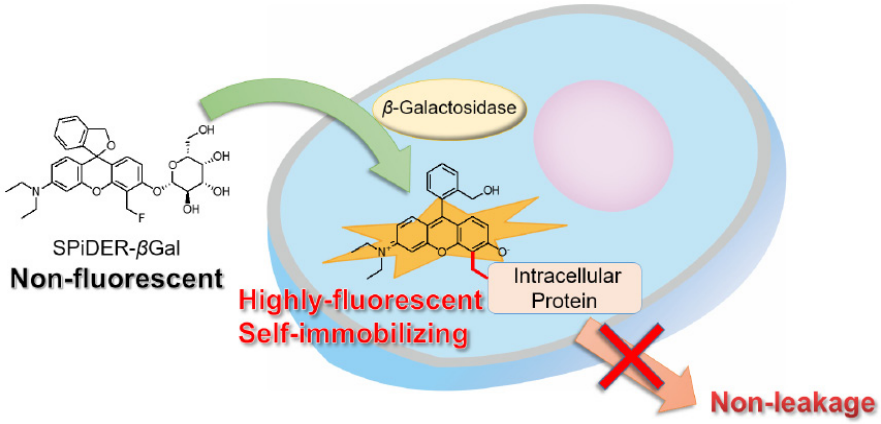
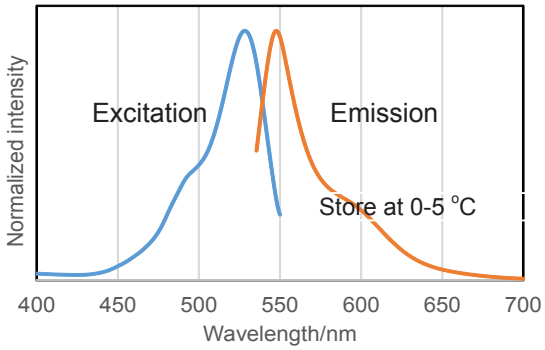


Fig. 1 Cell staining mechanism by SPiDER-βGal.



＜ Recommended filter ＞
Ex：500 ～ 540 nm
Em：530 ～ 570 nm
There are internal detection examples obtained using confocal microscopy and flow cytometry at an excitation wavelength of 488 nm.

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

After a SPiDER-βGal is taken out from the seal bag, keep the unused SPiDER-βGal in the bag, seal tightly and 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)

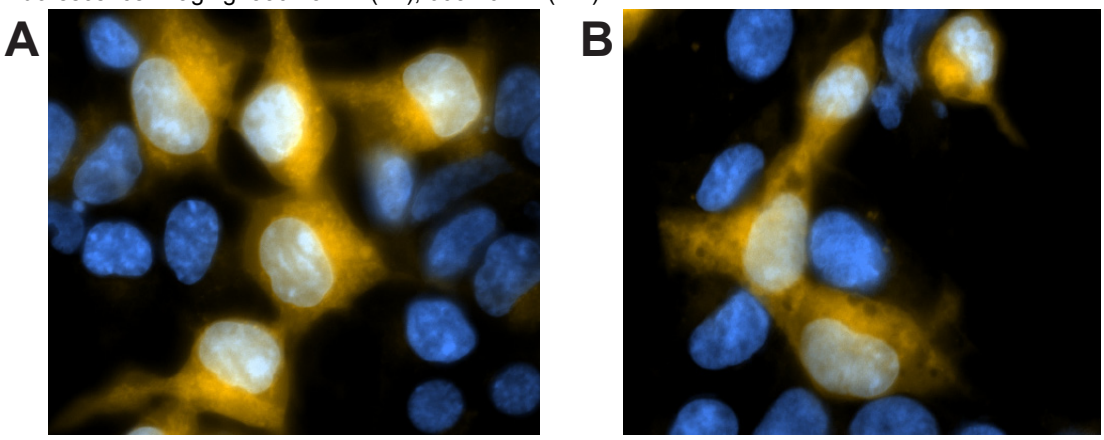


Fig. 3 Fluorescence imaging of HEK/LacZ cells and HEK cells at 1: 1 ratio.
A. living cell, B. fixing cells (4% PFA/PBS)
(yellow: SPiDER-βGal, blue: Hoechst 33342)

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)

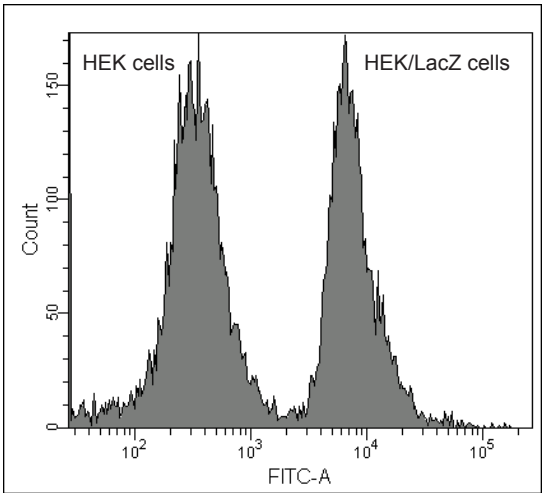


Fig. 4 Analysis of HEK/LacZ cells and HEK cells at 1: 1 ratio by flow cytometry.

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

Reference

1) T. Doura, M. Kamiya, F. Obata, Y. Yamaguchi, T. Y. Hiyama, T. Matsuda, A. Fukamizu, M. Noda, M. Miura and Y. Urano, *Angew. Chem. Int. Ed.*, **2016**, *55*, 9620.

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