Cellular Senescence Detection Kit - SPiDER-βGal

Technical Manual

General Information

DNA damages in normal cells are caused by repeated cell division and oxidative stress. Cellular Senescence, a state of irreversible growth arrest, can be triggered in order to prevent DNA-damaged cells from growing. Senescence-associated β-galactosidase (SA-β-gal), which is overexpressed in senescent cells, has been widely used as a marker of cellular senescence. Although X-gal staining is widely used to detect SA-β-gal, this method has the following disadvantages: 1) requirement of fixed cells due to the poor cell-permeability, 2) low quantitative capability because of the difficulty of discrimination between stained cells and unstained cells, 3) requirement of a long time for staining.

Cellular Senescence Detection Kit - SPiDER-βGal allows to detect SA-β-gal with high sensitivity and ease of use. SPiDER-βGal is a new reagent to detect β-galactosidase, and possesses high cell-permeability and intracellular retentivity. SA-β-gal in living cells can be specifically detected by using a reagent, Bafilomycin A1, to inhibit endogeneous β-galactosidase activity. In addition, SA-β-gal in fixed cells is also detectable by using McIlvaine buffer (pH 6.0). Since SPiDER-βGal emits strong and stable fluorescence after the reaction with SA-β-gal, it can be applied to quantitative analysis by flow cytometry.

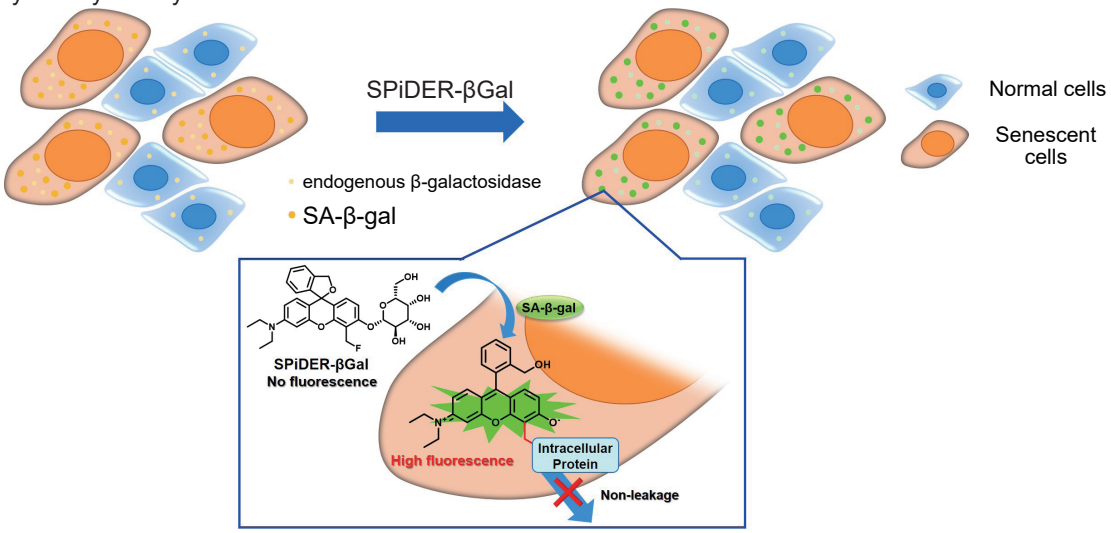
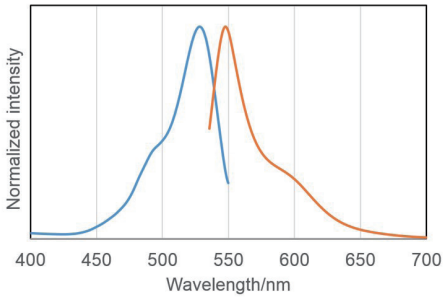


Fig. 1 Detection mechanism of senescent cells by SPiDER-βGal



＜ 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

Kit Contents

1 plate (6-well plate)
- SPiDER-βGal x 1
- Bafilomycin A1 x 1

※ The Bafilomycin A1 in the tube may be barely visible due to the small amount. Please handle it carefully.

Storage Condition

Store at 0-5°C

Required Equipment and Materials

- Dimethyl sulfoxide (DMSO)
- Culture medium or HBSS
- Micropipettes

Preparation of Solutions

Preparation of SPiDER-βGal DMSO stock solution

Add 7 µl of DMSO to a tube of SPiDER-βGal and dissolve it with pipetting.
Store the SPiDER-βGal DMSO stock solution at -20°C.

Preparation of Bafilomycin A1 DMSO stock solution

Add 24 µl of DMSO to a tube of Bafilomycin A1 and dissolve it with pipetting.
Store the Bafilomycin A1 DMSO stock solution at -20°C.

※ Insoluble substances such as lipofuscin accumulate in cells according to the acceleration of cellular senescence. Because lipofuscin has autofluorescence, it may results in higher fluorescence background. In order to achieve accurate SA-β-gal activity assay in senescent cells, we recommend preparing samples without SPiDER-βGal staining and compare the fluorescence intensities of both cells with or without SPiDER-βGal staining. Please refer to our web page or contact technical service for details.

General Protocol

— Assay for living cells — (for 6-well plate)

Preparation of Bafilomycin A1 working solution

Dilute the Bafilomycin A1 DMSO stock solution 1,000 times with culture medium or HBSS.

Preparation of SPiDER-βGal working solution

Mix the SPiDER-βGal DMSO stock solution and Bafilomycin A1 DMSO stock solution. Dilute the mixture 1,000 times with culture medium or HBSS.

※ For example, in order to prepare 1 ml of the SPiDER-βGal working solution, mix 1 µl of SPiDER-βGal DMSO stock solution and 1 µl of Bafilomycin A1 DMSO stock solution. Dilute the mixture with 1 ml of culture medium or HBSS.

1. Prepare cells in a 6-well plate for assay and culture the dish at 37°C overnight in a 5% CO_2 incubator.
2. Discard the culture medium and wash the cells with 2 ml of culture medium or HBSS once.
3. Add 1 ml of Bafilomycin A1 working solution and incubate at 37°C for 1 hour in a 5% CO_2 incubator.
4. Add 1 ml of SPiDER-βGal working solution and incubate at 37°C for 30 minutes in a 5% CO_2 incubator.
5. After removing the supernatant, wash the cells with 2 ml of culture medium or HBSS twice.
6. Observe the cells under a fluorescence microscope or analyze by a flow cytometer.

— Assay for fixed cells — (for 6-well plate)

Preparation of SPiDER-βGal working solution

Dilute the SPiDER-βGal DMSO stock solution 2,000 times with McIlvaine buffer (pH 6.0).

※ Preparation of McIlvaine buffer (pH 6.0):
Mix 0.1 mol/l citric acid solution (3.7 ml) and 0.2 mol/l sodium phosphate solution (6.3 ml). Confirm the pH is 6.0.
If the pH is not 6.0, adjust the pH by adding either citric acid solution or sodium phosphate solution. Dilute this buffer 5 times with ultrapure water.

1. Prepare cells in a 6-well plate for assay and culture the dish at 37°C overnight in a 5% CO_2 incubator.
2. After removing the culture medium, wash the cells with 2 ml of HBSS once. Add 2 ml of 4%paraformaldehyde (PFA) / PBS solution to the cells and incubate at room temperature for 3 minutes.
3. Remove the supernatant, and wash the cells with 2 ml of HBSS three times.
4. Add 2 ml of SPiDER-βGal working solution and incubate at 37°C for 30 minutes.
   ※ We recommend not to use a 5% CO_2 incubator for fixed cell experiments.
   If incubation is done in a 5% CO_2 incubator, the pH of the buffer may become acidic. Acidic pH results in higher background from the endogenous β-galactosidase activity and it would be difficult to distinguish between normal cells and senescent cells.
5. After removing the supernatant, wash the cells with 2 ml of HBSS twice.
6. Observe the cells under a fluorescence microscope or analyze by a flow cytometer.
   ※ Examples of a flow cytometry analysis are available in the product web page.

Usage Examples

Fluorescence imaging of SA-β-gal

1. WI-38 cells (5×10^4 cells/dish, MEM, 10%fetal bovine serum, 1%penicillin-streptmycin) of passage number 0 and 12 were seeded respectively in a µ-dish 35 mm (ibidi) and cultured overnight in a 5% CO_2 incubator.
2. The cells were washed with 2 ml of HBSS once.
3. Bafilomycin A1 working solution (1 ml) was added to the culture dish, and the cells were incubated for 1 hour in a 5% CO_2 incubator.
4. SPiDER-βGal working solution (1 ml) and 1 mg/ml Hoechst 33342 (1 µl) were mixed. Then the mixture solution was added to the culture dish, and the cells were incubated for 30 minutes in a 5% CO_2 incubator.
5. After the supernatant was removed, the cells were washed with 2 ml of HBSS twice.
6. HBSS (2 ml) were added and the cells were observed by confocal fluorescence microscopy (Excitation: 488 nm, Emission: 500-600 nm).

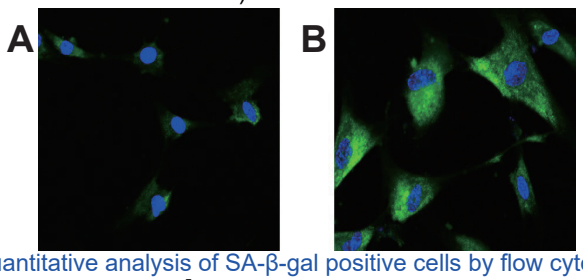


Fig. 3 Fluorescence imaging of SA-β-gal in WI-38 cells
A. Passage 0, B. Passage 12
(green: SPiDER-βGal, blue: Hoechst 33342)

Quantitative analysis of SA-β-gal positive cells by flow cytometry

1. WI-38 cells (1×10^5 cells/dish, MEM, 10%fetal bovine serum, 1% penicillin-streptmycin) of passage number 1 and 12 were seeded respectively in a µ-dish 35 mm (ibidi) and cultured overnight in a 5% CO_2 incubator.
2. The cells were washed with 2 ml of HBSS once.
3. Bafilomycin A1 working solution (1 ml) was added to the culture dish, and the cells were incubated for 1 hour in a 5% CO_2 incubator.
4. SPiDER-βGal working solution (1 ml) was added to the culture dish, and the cells were incubated at for 30 minutes in a 5% CO_2 incubator.
5. After the supernatant was removed, the cells were washed with 2 ml of HBSS twice.
6. The cells were harvested by trypsin and resuspended in MEM (10%fetal bovine serum, 1%penicillin-streptmycin).
7. The cells were observed by a flow cytometer (Excitation: 488 nm, Emission: 515-545 nm).

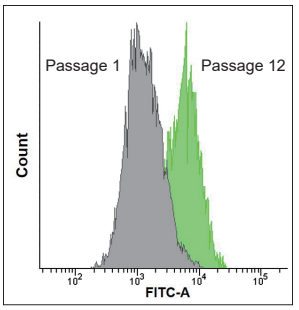


Fig. 4 Quantification of SA-β-gal positive WI-38 cells

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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