USER MANUAL

FastCellular™

Senescence Detection Kit - SPiDER-βGal

Cat. No. 092690301

Revision Date: 2021-02

PRODUCT DESCRIPTION

Cellular senescence is a phenomenon by which normal cells cease to divide and enter a state of irreversible growth arrest. It is one of the most fundamental aspects of cellular behavior and generally believed to be associated with tumor suppressive mechanisms and an underlying cause of aging. 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 is a well-known reagent used to detect SA-β-gal, X-gal has the following disadvantages: 1) requirement of fixed cells due to poor cell-permeability, 2) low quantitative capability due to difficulty in visually differentiating stained vs. non-stained cells, 3) requirement for long stain times.

To overcome the disadvantages inherent in using X-gal, we utilize a new reagent and method for the detection of cellular senescence (Figure 1). SPiDER-βGal offers increased sensitivity in detecting SA-β-gal due to increased cell permeability and retention inside cells. Compared to the X-gal method, the FastCellular™ Senescence Detection Kit can be used for both living and fixed cells.

Bafilomycin A1 is included in the kit to specifically inhibit endogenous β-galactosidase activity and thus increase the detection specificity for living cells. For fixed cells, SA-β-gal is detectable by using SPiDER-βGal and McIlvaine buffer (pH 6.0, please refer to the protocol for fixed cell assays). 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. In addition, the staining time is only 30 minutes.

For research use only.
Not for use in diagnostic procedures.

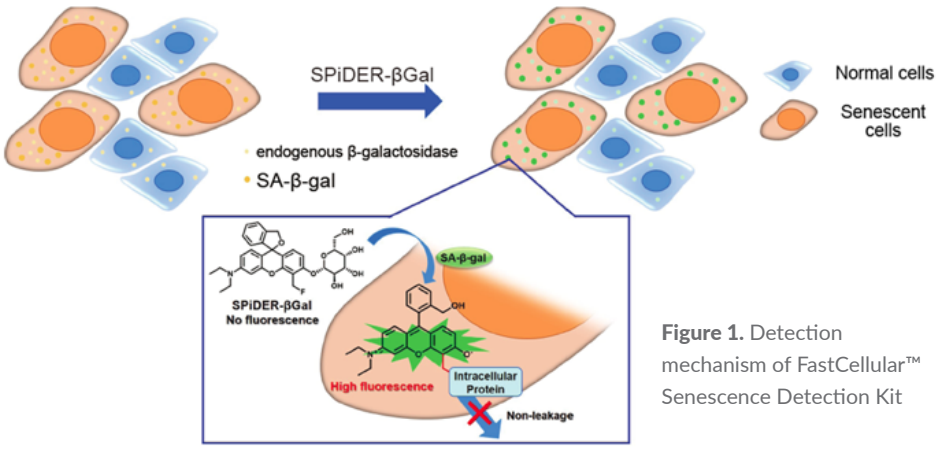

Figure 1. Detection mechanism of FastCellular™ Senescence Detection Kit

KEY BENEFITS

- **Higher sensitivity with a new fluorogenic detection probe**
- **Applicable for living cells and fixed tissues**
- **Ability to quantify SA-β-gal in cellular senescence assay**
- **Short staining time (30 min)**

KIT CONTENTS

One plate (6-well plate) with
1 unit of SPiDER-βGal and
1 unit of Bafilomycin A1.

STORAGE

Store at 0-5 °C with protection from light.

STOCK SOLUTION PREPARATION

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.

GENERAL CELLULAR SENESCENCE DETECTION PROTOCOL

Assay for living cells (6-well plate) (Figure 2)

1. 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 at equal ratios. Dilute the mixture 1,000 times with culture medium or HBSS.
2. Prepare cells in a 6-well plate for assay and culture the dish at 37 °C overnight in a 5% CO_2 incubator.
3. Discard the culture medium and wash the cells with 2 mL of culture medium or HBSS once.
4. Add 1 mL of Bafilomycin A1 working solution and incubate at 37 °C for 1 hour in a 5% CO_2 incubator.
5. Add 1 mL of SPiDER-βGal working solution and incubate at 37 °C for 30 minutes in a 5% CO_2 incubator.
6. After removing the supernatant, wash the cells with 2 mL of culture medium or HBSS twice.
7. Observe the cells under a fluorescence microscope (Figure 3. Ex: 500~540 nm, Em: 530~570 nm) or analyze by a flow cytometer.

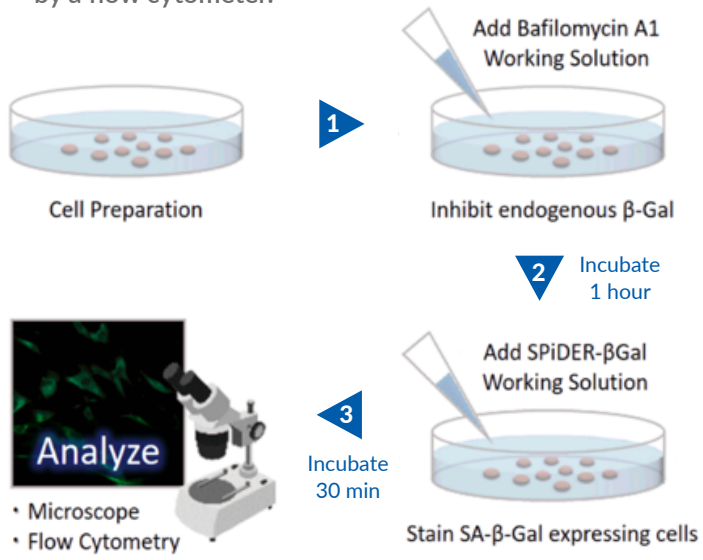

Figure 2. General cellular senescence detection procedure for living cells.

Assay for fixed cells (6-well plate)

1. 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.
2. Prepare cells in a 6-well plate for assay and culture the dish at 37 °C overnight in a 5% CO_2 incubator.
3. 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.
4. Remove the supernatant and wash the cells with 2 mL of HBSS three times.
5. 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 due to possible pH changes of the buffer during incubation.
6. After removing the supernatant, wash the cells with 2 mL of HBSS twice.
7. Observe the cells under a fluorescence microscope (Figure 3) or analyze by a flow cytometer.

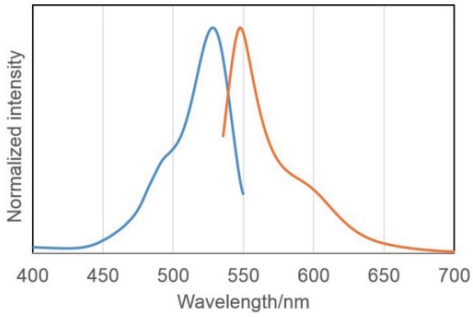

Figure 3. Excitation and emission spectra of SPiDER-βGal after reaction with β-galactosidase (Ex: 500~540 nm, Em: 530~570 nm. You may use an excitation wavelength of 488 nm for confocal microscopy.)