

Protocol for SPiDER- β Gal Cellular Senescence Dye (Catalog # 9034)

In Brief

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 requires to fix cells or tissues due to the poor cell-permeability. In addition, assays using fluorescence reagents cannot clearly differentiate β -galactosidase-expressed cells or regions.

To overcome these issues, Urano, Kamiya and co-workers have successfully developed SPiDER- β Gal. SPiDER- β Gal ideally possesses cell-permeability and the ability to retain in intracellular region¹.

Upon reaction with the enzyme, SPiDER- β Gal immediately forms a quinone methide that acts as electrophile when proteins containing nucleophilic functional groups are nearby the molecules. When the probe undergoes the reaction with a protein, the conjugates become fluorescent and remain inside cells by anchoring to intracellular proteins and provides single-cell resolution.

1. Required Equipment and Materials

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

2. Preparation of the Solutions

Preparation of 1 mmol/l SPiDER- β Gal DMSO stock solution

Add 35 μ l of DMSO to a tube of SPiDER- β Gal (20 μ g) and dissolve it with pipetting.

Note: Store the SPiDER- β Gal stock solution at -20°C.

Preparation of 1 μ mol/l SPiDER- β Gal working solution

Dilute the SPiDER- β Gal DMSO stock solution with Hanks' HEPES buffer to prepare 1 μ mol/l SPiDER- β Gal working solution.

Note: Hanks' HEPES buffer is recommended to maintain cell condition.

3. General Protocol

SPiDER- β Gal 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- β Gal working solution.
4. Incubate at 37°C for 15 minutes.
5. Observe the cells under a fluorescence microscope or by a flow cytometer.

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

4. Experimental Examples

4.1 Fluorescence microscopic detection of β -galactosidase-expressed cells

1. HEK cells at 5×10^5 cells/ml (500 μ l) and HEK/LacZ cells at 5×10^5 cells/ml (500 μ l) were seeded in a 35 mm dish in DMEM (10% fetal bovine serum, 1% penicillin-streptomycin) and cultured overnight in a 5% CO₂ incubator at 37°C.
2. The cells were washed with 2 ml of Hanks' HEPES buffer twice.
3. SPiDER- β Gal 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.
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.

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

4.2 Flow cytometric detection of β -galactosidase-expressed cells

1. HEK cells at 5×10^5 cells/ml (500 μ l) and HEK/LacZ cells at 5×10^5 cells/ml (500 μ l) were mixed in a microtube.
2. SPiDER- β Gal DMSO stock solution (1 μ l) 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)

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

5. References

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.