Cellular Senescence Plate Assay Kit - SPiDER-βGal

Technical Manual

Technical Manual (Japanese version) is available at http://www.dojindo.co.jp/manual/SG05.pdf

General Information

DNA damage in normal cells is caused by repeated cell division and oxidative stress. Cellular senescence, a state of irreversible growth arrest, can be triggered to prevent DNA-damage. Senescence-associated β-galactosidase (SA-β-gal), which is overexpressed in senescent cells, has been widely used as a marker of cellular senescence [1, 2].
The kit enables simple determination of cellular senescence by measuring SA-β-gal activity using a fluorometric substrate, SPiDER-βGal [3]. The assay system can be combined with widely used normalization methods (e.g. using a hemocytometer, BCA assay, and nucleic acid stains).

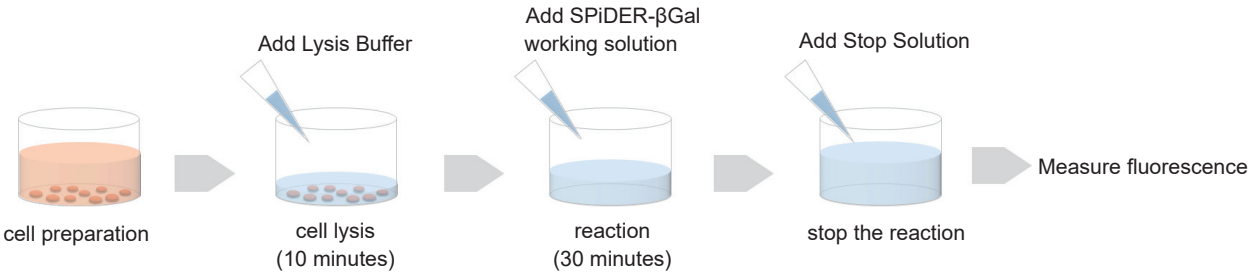

Figure 1 Assay procedure

Kit Contents

	20 tests	100 tests
SPiDER-βGal	1 tube	5 tubes
Lysis Buffer	40 mL×1	100 mL×2
Assay Buffer	1.5 mL×1	7.5 mL×1
Stop Solution	3 mL×1	15 mL×1

Storage Condition

Store at 0–5 °C and protect from light.

Required Equipment and Materials

– Fluorometer
– 96-well black plate
– Incubator (37 °C)
– Multi-channel pipette (20–200 µL)
– Micropipette (100–1000 µL, 20–200 µL)
– Phosphate buffered saline (PBS)
– Dimethylsulfoxide (DMSO)
– Conical tubes

Precautions

- Equilibrate reagents to room temperature prior to use.
- Centrifuge the tube (SPiDER-βGal) briefly before opening to remove all contents from the tube walls and inside the cap.
- Analyzing samples in triplicate is recommended for accuracy.
- Because the coloration reaction starts immediately after addition of the working solution to a well, use a multi-channel pipette to minimize experimental error by reducing the pipetting steps.

Preparation of Solutions

Preparation of SPiDER-βGal DMSO stock solution

Add 125 µL DMSO to the SPiDER-βGal tube and dissolve the contents using a vortex mixer.

*SPiDER-βGal is difficult to see by the naked eye because of the small amount.
*Vortexing is necessary to completely dissolve the contents (SPiDER-βGal).
*Store the SPiDER-βGal DMSO stock solution at -20 °C and protect from light (insert the bag). The prepared stock solution is stable at -20 °C for 1 month.

Preparation of SPiDER–βGal working solution

Prepare a 10-fold dilution of the stock solution in Assay Buffer.

*Prepare the working solution fresh each day.
*When using a 96-well plate, 50 µL of working solution is needed for each well.
*The working solution is light-sensitive. Protect it from light by covering the tube with aluminum foil.

General Protocol

SA-β-gal assay

1. Seed cells on a plate or dish and culture at 37 °C overnight in a 5% CO_2 incubator.
2. Perform suitable normalization for your experiment.
   *If you need any assistance for normalization, please contact Dojindo's technical support.
3. Remove the supernatant and wash the cells with PBS once.
4. Add Lysis Buffer and incubate the plate or dish at room temperature for 10 minutes.
   *For the amount of Lysis Buffer, please refef to Table 1

	96-well plate	24-well plate	6-well plate	10-cm dish
Lysis Buffer	50 µL	400 µL	1 mL	1.5 mL

Table 1 Lysis Buffer amount to be added

5. Transfer 50 µL lysate solution to each well of a 96-well black plate.
6. Add 50 µL SPiDER–βGal working solution to each well and incubate at 37 °C for 30 minutes.
   *Incubation time can be extended if necessary.
7. Add 100 µL Stop Solution to each well.
8. Measure fluorescence using a fluorometer (Ex: 500–540 nm; Em: 540–580 nm).

Experimental Examples

Determination of SA-β-gal in WI-38 cells

1. Passage 3 and 19 WI-38 cells (1×10^4 cells/well, MEM containg 10% fetal bovine serum and 1% penicillin-streptomycin) were seeded on a 96-well black plate (clear bottom) and cultured at 37 °C overnight in a 5% CO_2 incubator.
2. Cell Count Normalization Kit (code: C544) was used for normalization, which is a nucleoic acid stain based normalization kit.
   *For this kit, more infomation is available at our web-site.
3. The supernatant was removed and the cells were washed with 100 µL PBS once.
4. After addition of 50 µL Lysis Buffer to each well, the plate was incubated at room temperature for 10 minutes.
5. SPiDER-βGal working solution (50 µL) was added to each well and the plate was incubated at 37 °C for 30 minutes.
6. Stop Solution (100 µL) was added to each well.
7. Fluorescence signals were measured using a fluorometer (Ex: 535 nm; Em: 580 nm).
8. Normalized SA-β-Gal activity was determined using the Cell Count Normalization Kit.

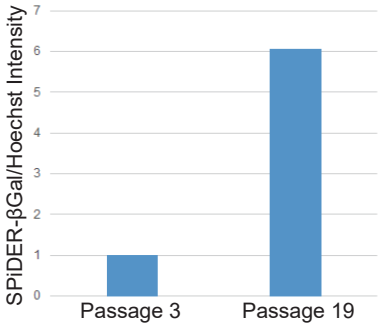

Figure 2 SA-β-gal activity in senescent WI-38 cells (microplate assay)

Determination of SA-β-gal in doxorubicin-treated WI-38 cells

1. Passage 3 WI-38 cells (1×10^6 cells/dish, MEM containing 10% fetal bovine serum and 1% penicillin-streptomycin) were seeded on a 10-cm dish and cultured at 37 °C overnight in a 5% CO_2 incubator.
2. The medium was removed and the cells were washed with 10 mL PBS once.
3. Doxorubicin solution (0.2 µmol/L in serum-free MEM) was added to the cells and the cells were cultured at 37 °C for 3 days in a 5% CO_2 incubator.
4. The supernatant was removed and the cells were washed with 10 mL PBS once.
5. MEM (containg 10% fetal bovine serum and 1% penicillin-streptomycin) was added and the cells were cultured at 37 °C for 3 days in a 5% CO_2 incubator.
6. The medium was removed and the cells were washed with 10 mL PBS once.
7. The doxorubicin-treated WI-38 cells (1×10^4 cells/well, MEM containg 10% fetal bovine serum and 1% penicillin-streptomycin) were seeded on a 96-well black plate (clear bottom) and cultured at 37 °C overnight in a 5% CO_2 incubator.
8. The doxorubicin-untreated WI-38 cells (1×10^4 cells/well, MEM containg 10% fetal bovine serum and 1% penicillin-streptomycin) were seeded on a 96-well black plate (clear bottom) and cultured at 37 °C overnight in a 5% CO_2 incubator as control.
9. Cell Count Normalization Kit (code: C544) was used for normalization, which is a nucleoic acid stain based normalization kit.
10. The supernatant was removed and the cells were washed with 100 µL PBS once.
11. After addition of 50 µL Lysis Buffer to each well, the plate was incubated at room temperature for 10 minutes.
12. SPiDER-βGal working solution (50 µL) was added to each well and the plate was incubated at 37 °C for 30 minutes.
13. Stop Solution (100 µL) was added to each well.
14. Fluorescence signals were measured using a fluorometer (Ex: 535 nm; Em: 580 nm).
15. Normalized SA-β-Gal activity was determined using the Cell Count Normalization Kit.

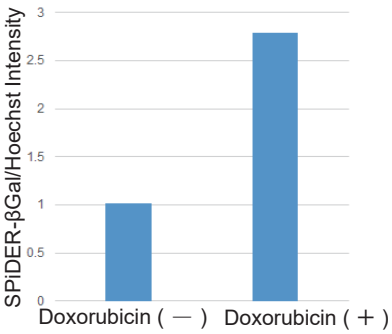

Figure 3 SA-β-gal activity in senescent WI-38 cells (microplate assay)

References

1) Dimri, G. P. et al., *Cell Biology*, **1995**, *92*, 9363–9367.
2) Park, A. M. et al., *J. Biol. Chem*., **2018**, *293*, DOI: 10.1074/jbc.RA118.003310
3) Doura, T. et al., *Angew. Chem*., **2016**, *55*, 9620–9624.