Store at -20°C & RT

#23833

Senescence β-Galactosidase Activity Assay Kit (Fluorescence, Plate-Based)

1 Kit
(100 assays)

Cell Signaling TECHNOLOGY®

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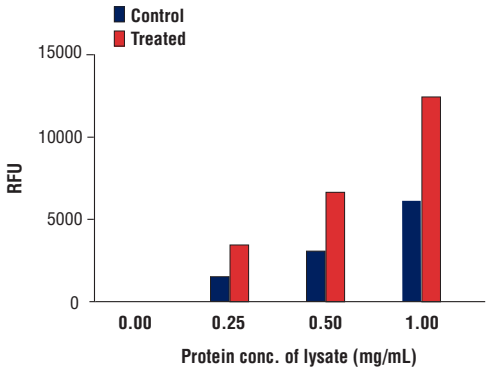
For Research Use Only. Not For Use In Diagnostic Procedures.

Description: The Senescence β-Galactosidase Activity Assay Kit (Fluorescence, Plate-Based) uses the β-gal Substrate 4-Methylumbelliferyl β-D-galactopyranoside (4-MUG) to detect Senescence-Associated beta-galactosidase (SA-β-gal) activity. Upon binding to β-gal, 4-MUG is hydrolysed to the fluorescent product 4-MU that can be measured at an excitation wavelength of 360 nm and an emission wavelength of 465 nm. Fluorescent intensity correlates with sample β-gal levels. This quantitative assay uses cellular lysates for determination of SA-β-gal activity. Each kit provides enough reagents to perform up to 100 assays in a 96-well plate format.

Background: β-galactosidase (also known as β-gal) is an essential hydrolase enzyme that catalyzes the hydrolysis of galactose-containing carbohydrates into monosaccharides. Substrates of β-galactosides include lactose, various glycoproteins, ganglioside GM1, and lactosylceramides. β-galactosidase is used widely in molecular biology; for example, isolation of recombinant bacteria during molecular cloning utilizes α-complementation of the bacterial β-galactosidase gene (*lacZ*) in the presence of a β-gal substrate to identify recombinant clones (1). In cell biology, Senescence-Associated beta-galactosidase (SA-β-gal), defined as β-gal activity at pH 6.0, is a widely used marker of replicative senescence. While initially thought to derive from a unique isoform of β-galactosidase expressed specifically in senescent cells (2), SA-β-gal activity was subsequently shown to result from overexpression and accumulation of β-galactosidase in endogenous lysosomes, and is not specifically required for replicative senescence (3).

Products Included	Item #	Quantity	Storage Temp
Senescence 1X Cell Lysis Buffer	91029	25 mL	RT
Senescence 2X Reaction Buffer	78494	6.5 mL	RT
Senescence Stop Solution	66008	21 mL	RT
SA-β-Gal Substrate	45954	4 mg	-20°C



The relationship between lysate protein concentration from HeLa cells, untreated and treated with Doxorubicin #5927 (200 nM, 24 hr, then rest for 3 days in fresh growth media), and the Relative Fluorescence Units (RFU) as determined by the Senescence β-Galactosidase Activity Assay Kit (Fluorescence, Plate-Based) is shown.

Storage: All components in this kit are stable for at least 12 months when stored at the recommended temperature.

Background References:

(1) Messing, J. et al. (1977) *Proc Natl Acad Sci U S A* 74, 3642-6.

(2) Dimri, G.P. et al. (1995) *Proc Natl Acad Sci U S A* 92, 9363-7.

(3) Lee, B.Y. et al. (2006) *Aging Cell* 5, 187-95.

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Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity:** **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

Senescence β-Galactosidase Activity Assay Kit (Fluorescence, Plate-Based) Protocol

A Solutions and Reagents

Supplied Reagents

1. 1X Senescence Cell Lysis Buffer: #91029
2. 2X Senescence Reaction Buffer: #78494
3. SA-β-Gal Substrate: #45954
   Reconstitute vial of SA-β-Gal Substrate in 348 µL of DMSO to make a 20X solution. Store any unused substrate, protected from light, at -20°C for up to 1 month from date of reconstitution.
4. Senescence Stop Solution: #66008

Additional Reagents (Not Supplied)

1. β-mercaptoethanol (BME)
2. DMSO (Dimethyl Sulfoxide) Sterile: (#12611)
3. PMSF: (#8553) Reconstitute 34.84 mg of lyophilized PMSF in 1.0 ml isopropyl alcohol, to make a 200 mM solution.
4. Protease/Phosphatase Inhibitor Cocktail (100X): (#5872)
5. Phosphate Buffered Saline (PBS-20X): (#9808) To prepare a 1X solution, add 0.5 mL of 20X PBS to 9.5 mL of deionized water.
6. 96-well plate for initial reaction found in Section C. Test Procedure Step 1.
7. 96-well black opaque plate suitable for a fluorescent plate reader.
8. Plate reader capable of reading excitation at 360 nm and emission at 465 nm.
9. BCA Protein Assay Kit: (#7780)
10. High Speed Centrifuge

Reagent	96-well	24-well	6-well	10cm dish
1X PBS Wash	100 µl	400 µl	1000 µl	1500 µl
1X Cell Lysis Buffer	100 µl	400 µl	1000 µl	1500 µl

B Preparing Cell Lysates

NOTE: Cell treatments to induce senescence should be completed before initiating assay with this kit. Ensure that an untreated control is included, to provide a baseline measurement of fluorescence for comparison.

1. Plate cells of interest in a tissue culture vessel and culture under appropriate conditions used to generate senescent cells.
2. Prepare a 1X Senescence Cell Lysis Buffer immediately before use; add the proper amount of protease inhibitor such as 1.0 mM PMSF and protease/phosphatase inhibitor cocktail to the solution. Keep solution on ice while making the cell lysate. Refer to the provided table to calculate how much volume is needed based on the size of the cell culture vessel.
3. Aspirate the medium from the vessel containing cells being analyzed.
4. Wash the cells once with cold 1X PBS (refer to the table for correct volumes), then aspirate and discard the wash.
5. Add cold 1X Senescence Cell Lysis Buffer (refer to Section B. Step 2) to the cells (refer to the table for correct volumes). Incubate on ice for 5 minutes.
6. Scrape the cells off the surface and transfer the whole lysate to a micro-centrifuge tube. Pipette the lysate up and down several times to facilitate the lysing process.
7. Place the micro-centrifuge tubes containing the cell lysate into a centrifuge and spin for 5 minutes (x14000 rpm) at 4°C.
8. Transfer the supernatant to a new tube. The supernatant is the cell lysate. Store at -80°C in single-use aliquots.
9. Optional, determine the total protein concentration of each cell lysate sample by protein assay.

C Test Procedure

2X Assay Buffer Preparation: Immediately before use, add BME to 2X Senescence Reaction Buffer at a final concentration of 10 mM. Then dilute the SA-β-Gal Substrate to 1X with 2X Senescence Reaction Buffer containing 10mM BME. Make only enough solution for the number of wells analyzed (50 µL per well). Use the 2X Assay Buffer immediately and properly discard any unused solution.

NOTE: Protect the 2X Assay Buffer from light by wrapping with aluminum foil.

1. Transfer 50 µL of the cell lysate to a 96-well plate. Add 50 µL of freshly prepared 2X Assay Buffer. Incubate the samples at 37°C protected from light for 1-3 hours.
2. Remove 50 µL of the reaction mixture and transfer to a 96 well black opaque plate.
3. Stop the reaction by adding 200 µL of Senescence Stop Solution to each well.
4. Read fluorescence with a fluorescence plate reader set with excitation at 360nm and emission at 465nm. For optimal readings, read the plate within 30 minutes of adding STOP solution.