Store at -20°C

#35302

Senescence β-Galactosidase Activity Assay Kit (Fluorescence, Flow Cytometry)

1 Kit
(100 assays)

Support: +1-978-867-2388 (U.S.)
www.cellsignal.com/support

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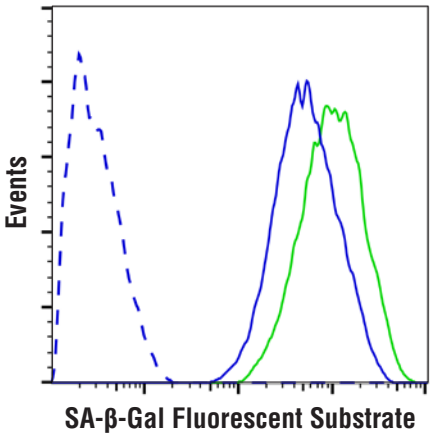
For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity
Bafilomycin A1	54645	33 µg
SA-β-Gal Fluorescent Substrate	38154	2 mg

Description: The Senescence β-Galactosidase Activity Assay Kit (Fluorescence, Flow Cytometry) allows the quantitative measure of senescence-associated β-galactosidase activity in living cells via flow cytometry using a cell permeable fluorogenic substrate, which becomes fluorescent upon hydrolysis by β-galactosidase. The fluorescent molecule is optimally excited at 490 nm (blue laser) and peak emission occurs at 514 nm (usually FL1). Each kit contains sufficient quantities of reagents to perform up to 100 assays.

Background: Limited capacity to replicate is a defining characteristic of most normal cells and culminates in senescence, an arrested state in which the cell remains viable (1). Senescent cells are not stimulated to divide by serum or passage in culture, and senescence invokes a specific cell cycle profile that differs from most damage-induced arrest processes or contact inhibition (2). An enlarged cell size, expression of pH-dependent β-galactosidase activity (3), and an altered pattern of gene expression (4,5) further characterize senescent cells.



Flow cytometric analysis of MCF7 cells, untreated (blue) or treated with Etoposide #2200 (12.5 µM, 24 hr; green) and allowed to recover for 3 days, using SA-β-Gal Fluorescent Substrate (solid lines) compared to unlabeled MCF7 cells (dashed line).

Storage: Store lyophilized or in solution at -20°C. In lyophilized form, the chemicals are stable for 24 months. Once in solution, use Bafilomycin A1 within three months, and use SA-β-Gal Fluorescent Substrate within one month.

Background References:

(1) Goldstein, S. (1990) *Science* 249, 1129-33.

(2) Sherwood, S. W. et al. (1988) *Proc. Natl. Acad. Sci., USA* 85, 9086-90.

(3) Dimri, G. et al. (1995) *Proc. Natl. Acad. Sci., USA* 92, 9363-7.

(4) Cristofalo, V. J. et al. (1998) *Crit. Rev. Eukaryot Gene Expr.* 8, 43-80.

(5) Linskens, M. H. et al. (1995) *Nucleic Acid Res.* 23, 3244-51.

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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.

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Senescence β-Galactosidase Activity Assay Kit (Fluorescence, Flow Cytometry)

A Solutions and Reagents

Supplied Reagents

1. **Bafilomycin A1 (#54645):** Prepare a 100 µM stock (1000X) by adding 530 µl DMSO. Once reconstituted, use within 3 months. Due to production methods, excess bafilomycin is provided. This material can be retained for use in other experiments or discarded.
2. **SA-β-Gal Fluorescent Substrate (#38154):** Prepare a 6.6 mM stock (200X) by adding 352 µl DMSO. Once reconstituted, use within 1 month.

Additional Reagents (Not Supplied)

1. Dimethyl Sulfoxide (DMSO) (#12611)
2. Phosphate Buffered Saline (PBS) (#12528)
3. Bovine Serum Albumin (BSA) (#9998)
4. 4% Formaldehyde, Methanol-Free (#47746) (optional)

B Detection of β-Galactosidase Activity

NOTE: Suggested dilutions and volumes are provided for reference. Optimal conditions for SA-β-Gal Fluorescent Substrate performance may vary by cell type and container type.

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

NOTE: Formaldehyde fixation may be performed at the completion of the incubation step with the SA-β-Gal Fluorescent Substrate #38154. Fixation with up to 4% formaldehyde for up to 15 minutes will not significantly reduce the fluorescence of the substrate. However, permeabilization with methanol or Triton X-100 will cause a significant reduction in fluorescence. If permeabilization is required for immunolabeling of intracellular targets, conduct an experiment to determine whether this will significantly compromise detection of the fluorescent substrate in that cell.

Analysis of Suspension Cells

1. Dilute the prepared 100 µM Bafilomycin A1 stock 1:1000 in the desired volume of culture medium, to produce a 100 nM solution. Use within 4 hrs.
2. Remove culture medium from cells through centrifugation. Add sufficient volume of Bafilomycin A1-containing medium to resuspend cells (roughly 1 mL per $1x10^6$ cells). Incubate at 37°C for 1 hr.
3. Add sufficient volume of the prepared 200X SA-β-Gal Substrate Solution directly to the cells in Bafilomycin A1-containing medium to produce a 1:200 dilution (33 µM). Gently mix cells to ensure even distribution of SA-β-Gal Substrate and incubate at 37°C for 2-4 hours.
4. Wash the stained cells three times with 3 mL of 1X PBS, through centrifugation.
5. Resuspend cells in cold PBS containing 2% FBS or 0.5% BSA.
6. (Optional) Perform immunostaining of live cells with antibodies against extracellular targets.
7. (Optional) Fix and permeabilize cells to perform immunostaining with antibodies against intracellular targets. See cautionary note above.
8. If necessary, obtain a cell count using a hemocytometer or other method, and collect an aliquot of the desired number of cells. Analyze the cells by flow cytometry.

Analysis of Adherent Cells

1. Dilute the prepared 100 µM Bafilomycin A1 stock 1:1000 in the desired volume of culture medium, to produce a 100 nM solution. Use within 4 hrs.
2. Remove culture medium from cells via aspiration. Add sufficient volume of Bafilomycin A1-containing medium to cover cells. Incubate at 37°C for 1 hr.
3. Add sufficient volume of the prepared 200X SA-β-Gal Substrate Solution directly to the cells in Bafilomycin A1-containing medium to produce a 1:200 dilution (33 µM). Gently mix cells to ensure even distribution of SA-β-Gal Substrate and incubate at 37°C for 2-4 hours.
4. Wash the stained cells three times with 3 mL of 1X PBS.
5. Remove PBS and trypsinize cells to remove from culture dish, per normal cell culture methods. Add media containing serum to inactivate trypsin. Collect cells, centrifuge and remove supernatant, and resuspend cells in cold PBS containing 2% FBS or 0.5% BSA.
6. (Optional) Perform immunostaining of live cells with antibodies against extracellular targets.
7. (Optional) Fix and permeabilize cells to perform immunostaining with antibodies against intracellular targets. See cautionary note above.
8. If necessary, obtain a cell count using a hemocytometer or other method, and collect an aliquot of the desired number of cells. Analyze the cells by flow cytometry.