

Version 3b, Last updated 4 July 2025

ab228562 Beta Galactosidase Assay Kit (Senescence)

For the measurement of Beta Galactosidase in cells.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Beta Galactosidase Assay Kit (Senescence) (ab228562) is designed to fluorescently detect Beta Galactosidase activity in cultured cells by FACS. The Beta Galactosidase is present only in senescent cells and is not found in pre-senescent, quiescent or immortal cells. Excitation and emission wavelength of the dye is 490 nm and 514 nm.

Culture cells for 48 hours with or without treatment.



Incubate cells in fresh media containing Senescence Dye



Analyse by flow cytometry

2. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt.

Aliquot components in working volumes before storing at the recommended temperature.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature
Assay Buffer 27	2 x 100 mL	-20°C
Senescence Dye	150 µL	-20°C

PLEASE NOTE: Assay Buffer 27 was previously labelled as Assay Buffer XXVII and Wash Buffer. The composition has not changed.

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Cell culture media
- 24-well plates
- Sterile dH₂O
- Green channel instruments (i.e. FACS FL-1)

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:

www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening under sterile conditions.

5.1 Assay Buffer 27

Store at -20°C or 4°C. Stable for six months after the first thaw. Bring to room temperature (RT) before use

5.2 Senescence Dye

Aliquot and store at -20°C. Avoid multiple freeze/ thaw. Thaw and keep on ice before use. Use within two months.

6. Assay Procedure – Suspension Cells

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate.

6.1 Cell culture:

1. Centrifuge cells at 700 x g, 4°C, 10 minutes and remove media.
2. Resuspend cell pellet in 500 µl of fresh medium (~10⁶ cells/ml) and transfer them to a 24 well plate. Treat cells with compounds of interest, if desired, for 48 hours at 37°C/5% CO₂.

6.2 Cell staining:

1. Post treatment, collect the cells in a sterile Eppendorf tube, centrifuge (700 x g, 4 °C, 10 min) to collect the cell pellet and remove the media.
2. Resuspend the cell pellet in 500 µl of fresh media containing 1.5 µl of Senescence Dye per tube. Incubate for 1-2 hr. at 37°C, 5% CO₂.
3. Wash 2X with 500 µl Assay Buffer 27. Resuspend cells in 500 µl Assay Buffer 27 and analyze immediately using flow cytometry.

6.3 FACS:

Signal should be measured in the FL1 channel. To ensure that only proper target cells are gated, use a side scatter versus FL-1 plot. We recommend keeping unstained control cells (i.e. without Dye staining) suspended in Assay Buffer 27 to set up the flow cytometer instrument.

7. Assay Procedure – Adherent Cells

7.1 Cell culture:

1. Seed cells ($\sim 5 \times 10^5$ cells per well) in a 24-well plate and incubate overnight.
2. Remove media, add fresh with compounds of interest, if desired, and treat for 48 hours at 37°C/5% CO₂. For control cells, we recommend treating cells with vehicle alone

7.2 Cell staining:

1. Remove the media and add fresh media containing 1.5 µl of Senescence Dye per 500 µl media. Incubate for 1-2 hr. at 37°C, 5% CO₂.
2. After incubation, wash the cells 2X with 500 µl Assay Buffer 27. Collect the cells by trypsinization, wash 1X with 500 µl and resuspend the cells in 500 µl Assay Buffer 27 and analyze immediately using flow cytometry.

7.3 FACS:

Signal should be measured in the FL1 channel. To ensure that only proper target cells are gated, use a side scatter versus FL-1 plot. We recommend keeping unstained control cells (i.e. without Dye staining) suspended in Assay Buffer 27 to set up the flow cytometer instrument.

8. Typical Data

Data provided for demonstration purposes only.

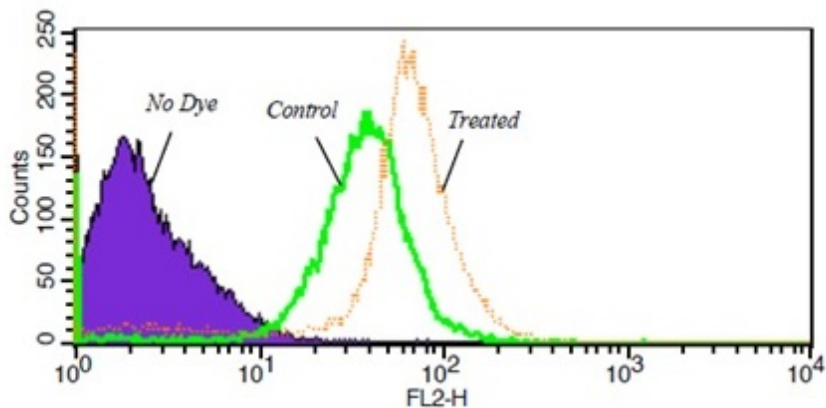


Figure 1. NIH/3T3 (mouse embryo fibroblast cell line) cells were plated at 5×10^5 cells per well in a 24 well, treated for 4 hours (with and without 200nM of Daunorubicin HCl; test and control response respectively) in complete cell culture media for 48 hours at 37°C/5% CO₂. Media was removed and replaced with media containing Senescence Dye and incubated for 2 hours at 37°C/5% CO₂. After incubation time, cells were washed 2x with Assay Buffer 27, the cells were trypsinized, washed once with Assay Buffer 27 and analyzed by FACS.

9. Notes

Technical Support

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