Senescence Detection Kit (Fluorometric) (BN01149)

(Catalog #BN01149; 100 assays; Store at -20°C)

I. Introduction:

Senescence is thought to be a tumor suppressive mechanism and an underlying cause of aging. Senescence represents an arrested state in which the cells remain viable, but not stimulated to divide by serum or passage in culture. Senescent cells display increase of cell size, senescence-associated expression of β-galactosidase (SA-β-Gal) activity and altered patterns of gene expression. The Senescence detection kit is designed to fluorescently detect SA-β-Gal activity in cultured cells by FACS. The SA-β-Gal is present only in senescent cells and is not found in pre-senescent, quiescent or immortal cells.

II. Applications:

- Detection of senescence in cells
- Screening potential senescence inducers/inhibitors

III. Sample Type:

- Adherent and suspension cells

IV. Kit Contents:

Components	BN01149	Cap Code
Wash Buffer	2 x 100 ml	NM
Senescence Dye	150 µl	Green

V. User Supplied Reagents and Equipment:

- Cell Culture Media
- Clear bottom, white walls, 24-well plates
- Sterile dH_2O
- Green Channel Instruments (*i.e.* FACS FL-1)

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening under sterile conditions. Read entire protocol before performing the assay. *Perform the assay under sterile conditions*.

- **Wash Buffer:** Store at -20°C or 4°C. Stable for six months after the first thaw. Bring to room temperature (RT) before use.
- **Senescence Dye:** Aliquot and store at –20°C. Avoid multiple freeze/ thaw. Thaw and keep on ice before use. Use within two months.

VII. Senescence Detection Assay Protocol:

The following protocol is designated for FACS experiments. Increase amounts and volumes accordingly if using a larger plate.

1. **Cell Culture:** *For suspension cells*: Centrifuge cells (700 x g, 4 °C, 10 min) and remove media. Resuspend cell pellet in 500 µl of fresh medium (~10^6 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% CO_2.

   For adherent cells: Seed cells (~$5x10^5$ cells per well) in a 24-well plate and incubate overnight. After incubation, remove media, add fresh media with compounds of interest, if desired, and treat for 48 hours at 37°C/5% CO_2. For control cells: we recommend treating cells with vehicle alone.

2. **Cell Staining**: *For Suspension cells*: 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. 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% CO_2 incubator. Wash 2X with 500 µl Wash buffer. Resuspend cells in 500 µl Wash buffer and analyze immediately using flow cytometry.

   For Adherent cells: 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% CO_2. After incubation, wash the cells 2X with 500 µl Wash buffer. Collect the cells by trypsinization, wash 1x with 500 µl and resuspend the cells in 500 µl Wash buffer and immediately analyze by flow cytometry.

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 Wash Buffer to set up the flow cytometer instrument.

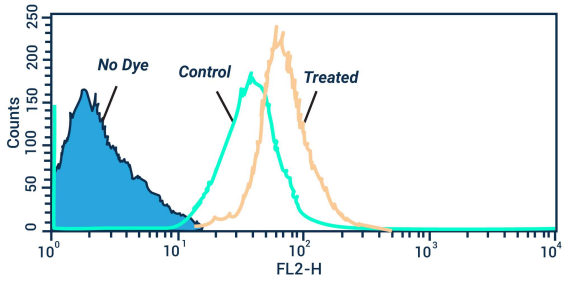

Figure: 3T3 cells were plated at $5x10^5$ cells per well in a 24well, treated for 4 hours (with and without 200nM of Daunorubicin. HCl; test and control response respectively) in complete cell culture media for 48 hours at 37°C/5% CO_2. Media was removed and replaced with media containing Senescence Dye and incubated for 2 hours at 37°C/5% CO_2. After incubation time, cells were washed 2x with Wash Buffer, the cells were trypsinized, washed once with Wash Buffer and analyzed by FACS.

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