Cell Meter™ Cellular Senescence Activity Assay Kit *Green Fluorescence*

Catalog number: 23005
Unit size: 100 Tests

Component	Storage	Amount (Cat No. 23005)
Component A: Xite™ beta-D-galactopyranoside	Freeze (< -15 °C), Minimize light exposure	1 vial
Component B: Assay Buffer	Freeze (< -15 °C)	1 bottle (20 mL)
Component C: DMSO	Freeze (< -15 °C)	1 vial (100 uL)

OVERVIEW

Cellular Senescence is an irreversible growth arrest triggered in order to prevent growth in DNA damaged cells. Senescence-associated beta-galactosidase (SA-beta-gal) is highly overexpressed in senescent cells and it has been widely used as a senescence marker. X-gal staining, a colorimetric method is widely available and used to detect SA-beta-gal in senescent cells. The color method has some limitations such as requirement of fixation of samples due to the low cell permeability of X-gal, longer staining time and low sensitivity. Cell Meter™ Cellular Senescence Activity Assay Kit uses Xite™ beta-D-galactopyranoside, a fluorogenic beta-Gal substrate that readily enters into live cells, and gets cleaved by SA-β-gal inside cells, generating strong green fluorescence. Unlike cell-impermeable X-Gal substrate, it has excellent cell permeability. Cell Meter™ Cellular Senescence Activity Assay Kit enables users to detect the senescence with higher sensitivity with robust performance. The Xite product is well retained inside the cells, producing a stable signal for fluorescence imaging and flow cytometry analysis.

AT A GLANCE

Protocol Summary

1. Treat samples as desired
2. Prepare and add Xite™ beta-D-galactopyranoside working solution to samples
3. Incubate samples at 37 °C for 15 to 45 minutes
4. Monitor the fluorescence intensity using flow cytometer with 530/30 nm filter (FITC channel)

Important Note

Thaw each kit component at room temperature before starting the experiment.

KEY PARAMETERS

Flow cytometer

Emission	530/30 nm filter
Excitation	488 nm laser
Instrument specification(s)	FITC channel

Fluorescence microscope

Emission	FITC filter set
Excitation	FITC filter set
Recommended plate	Black wall/clear bottom

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles

Xite™ beta-D-galactopyranoside stock solution (100X)

Add 100 uL DMSO (Component C) into Xite™ beta-D-galactopyranoside (Component A) and mix well. **Note:** Store the unused Xite™ beta-D-galactopyranoside stock solution at -20 °C in single use aliquots.

PREPARATION OF WORKING SOLUTION

Xite™ beta-D-galactopyranoside working solution (1X)

Dilute 10 uL of Xite™ beta-D-galactopyranoside stock solution (100X) with 1 mL of Assay Buffer to make Xite™ beta-D-galactopyranoside working solution (1X). **Note:** Xite™ beta-D-galactopyranoside working solution should be used promptly.

SAMPLE EXPERIMENTAL PROTOCOL

1. Treat your samples as desired.

   Note: For 96 well plate format, grow cells in 100 µL of cell culture medium and treat as desired. Volumes can be adjusted based on the plate size.

2. Wash the cells with buffer of your choice such as DPBS.

   Note: For selectively tracking β-Gal in live cells, cells can be treated with Bafilomycin A1 for blocking endogenous β-Gal. Optimum concentration of Bafilomycin A1 may vary on type of cells.

3. Add 100 µL Xite™ beta-D-galactopyranoside working solution for 15-45 minutes and incubate the samples at 37°C incubator.

   Note: Optimal time for incubation needs to be determined carefully.

4. Remove the working solution and wash cells with buffer of your choice.

   Note: If performing flow cytometry for attached cells, cells can be trypsined at this step and collected in a tube.

5. Resuspend the cells in the Assay Buffer (Component B) and monitor the fluorescence intensity with flow cytometer using 530/30 nm filter (FITC channel) or fluorescence microscope with FITC filter set.

EXAMPLE DATA ANALYSIS AND FIGURES

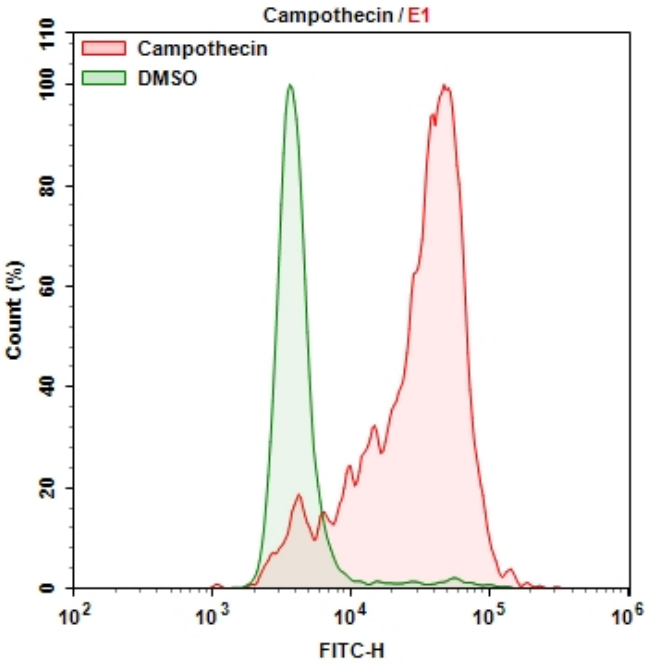

Figure 1. Cellular senescence was measured with Cell Meter™ Cellular Senescence Activity Assay Kit using a NovoCyte Flow Cytometer (ACEA Biosciences). HL-60 cells were incubated with Camptothecin for 6 hours to induce senescence and stained with Xite™ beta-D-galactopyranoside for 30 mins at 37^{o}C. The signal was acquired using FITC channel in ACEA NovoCyte flow cytometer.

DISCLAIMER

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