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

Catalog number: 23007
Unit size: 100 Tests

Component	Storage	Amount (Cat No. 23007)
Component A: Xite™ Red beta-D-galactopyranoside	Freeze (< -15 °C), Minimize light exposure	1 vial
Component B: Assay Buffer	Refrigerated (2-8 °C)	1 bottle (20 mL)
Component C: DMSO	Refrigerated (2-8 °C)	1 vial (100 µL)

OVERVIEW

Cellular Senescence is an irreversible growth arrest triggered in order to prevent growth in DNA damaged cells. Senescence-associated beta-galactosidase is highly overexpressed in senescent cells and has been widely used as a senescence marker. The colorimetric X-gal staining method is widely used to detect SA-beta-gal in senescent cells. However, the color method has some limitations such as the requirement of fixation of cells (due to the low cell permeability of X-gal), longer staining time and low sensitivity. Cell Meter™ Cellular Senescence Activity Assay Kit uses Xite™ Red beta-D-galactopyranoside, a fluorogenic beta-Gal substrate that readily enters into live cells, and gets cleaved by beta-galactosidase inside cells, generating strong red fluorescence. Unlike cell-impermeable X-Gal substrate, it has excellent cell permeability. The robust Cell Meter™ Cellular Senescence Activity Assay Kit enables users to detect the senescence with higher sensitivity. Xite™ Red beta-D-galactopyranoside is fixable for further cell analysis if desired. The red fluorescence of Xite™ Red can be readily combined with other color fluorescent probes such as DAPI or GFP. The Xite Red product is well retained inside 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™ Red beta-D-galactopyranoside working solution to samples
3. Incubate samples at 37 °C for 15 to 45 minutes
4. Monitor the fluorescence intensity with fluorescence microscope using Cy3/TRITC filter set or flow cytometer using 575/26 nm filter (PE channel)

Important

To ensure accurate results, allow all kit components to reach room temperature before beginning the experiment.

KEY PARAMETERS

Flow cytometer

Emission	575/26 nm filter
Excitation	488 nm laser
Instrument specification(s)	PE channel

Fluorescence microscope

Emission	Cy3/TRITC filter set
Excitation	Cy3/TRITC 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™ Red beta-D-galactopyranoside Stock Solution (100X)

Add 100 µL of DMSO (Component C) into Xite™ Red beta-D-galactopyranoside (Component A) and mix thoroughly.

Note: Divide any remaining Xite™ Red beta-D-galactopyranoside stock solution into individual single-use aliquots. Store these aliquots at -20°C, protected from light.

PREPARATION OF WORKING SOLUTION

Xite™ Red beta-D-galactopyranoside Working Solution

To prepare the Xite™ Red beta-D-galactopyranoside working solution, mix 10 µL of the Xite™ Red beta-D-galactopyranoside stock solution with 1 mL of Assay Buffer.

Note: Xite™ Red 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. Remove the cell culture medium and wash the cells with a 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. The optimum concentration of Bafilomycin A1 may vary on the type of cells.

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

   Note: The 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 a flow cytometer using a 575/26 nm filter (PE channel) or fluorescence microscope using a Cy3/TRITC filter set.

EXAMPLE DATA ANALYSIS AND FIGURES

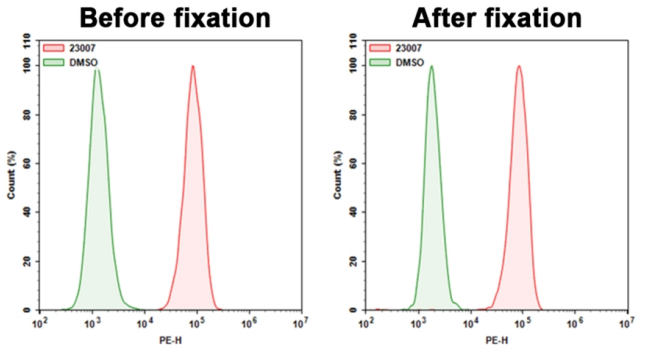

Figure 1. Fixability test with Cell Meter™ Cellular Senescence Activity Assay Kit using a NovoCyte Flow Cytometer (ACEA Biosciences). 9L-LacZ cells were incubated with DMSO or Xite™ Red beta-D-galactopyranoside for 45 mins at 37 °C. The signal before and after fixation was acquired using PE channel. (Cells were then fixed with 4% formaldehyde for 20 minutes at room temperature, and wash once.)

DISCLAIMER

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