Xite™ Red beta-D-galactopyranoside

Catalog number: 14035
Unit size: 1 mg

Component	Storage	Amount
Xite™ Red beta-D-galactopyranoside	Freeze (< -15 °C), Minimize light exposure	1 mg

OVERVIEW

Xite™ Red beta-D-galactopyranoside provides a simple and sensitive tool to detect beta-galactosidase (β-gal) activity. Compared to the existing red beta-galactosidase substrates (e.g., the commonly used resorufin beta-D-galactopyranoside), it has much better cell permeability. Xite™ Red beta-D-galactopyranoside provides a simple and sensitive tool to detect beta-galactosidase activity. Xite™ Red beta-D-galactopyranoside might be used as a simple tool for measuring cellular senescence in cells since β-gal has been identified as a reliable marker for cellular senescence. Xite™ Red beta-D-galactopyranoside enters readily cells where it gets cleaved by β-gal, producing Xite™ Red, a strongly fluorescent product. The strongly fluorescent Xite™ Red is well retained in cells, making it easy to be detected with a flow cytometer and fluorescence microscope. In addition, Xite™ Red beta-D-galactopyranoside is fixable. The red fluorescence generated by Xite™ Red beta-D-galactopyranoside can be readily combined with other color fluorescent probes such as DAPI or GFP for multicolor fluorescence 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 using flow cytometer with 575/26 nm filter (PE channel) or using fluorescence microscopy with Cy3/TRITC filter set

KEY PARAMETERS

Flow cytometer

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

Fluorescence microscope

Excitation	Cy3/TRITC filter set
Emission	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
Add appropriate amount of DMSO into Xite™ Red beta-D-galactopyranoside to make 2-5 mM Xite™ Red beta-D-galactopyranoside stock solution. **Note:** Store the unused Xite™ Red beta-D-galactopyranoside stock solution at -20 °C in single use aliquots.

PREPARATION OF WORKING SOLUTION

Xite™ Red beta-D-galactopyranoside working solution

Prepare 1-20 μM of Xite™ Red beta-D-galactopyranoside working solution in buffer of your choice. **Note:** Xite™ Red beta-D-galactopyranoside working solution should be used promptly. **Note:** The concentration of the Xite™ Red beta-D-galactopyranoside should be optimized for different cell types and conditions.

SAMPLE EXPERIMENTAL PROTOCOL

The following protocol can be used as a guideline and should be optimized according to the needs.

1. Treat your samples as desired.
2. Remove the treatment and wash 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 Xite™ Red 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 experimentally.
4. Remove the working solution and wash cells with buffer of your choice.
5. Resuspend the cells in buffer of your choice and monitor the fluorescence intensity with flow cytometer using 575/26 nm filter (PE channel) or fluorescence microscope with Cy3/TRITC filter set.

EXAMPLE DATA ANALYSIS AND FIGURES

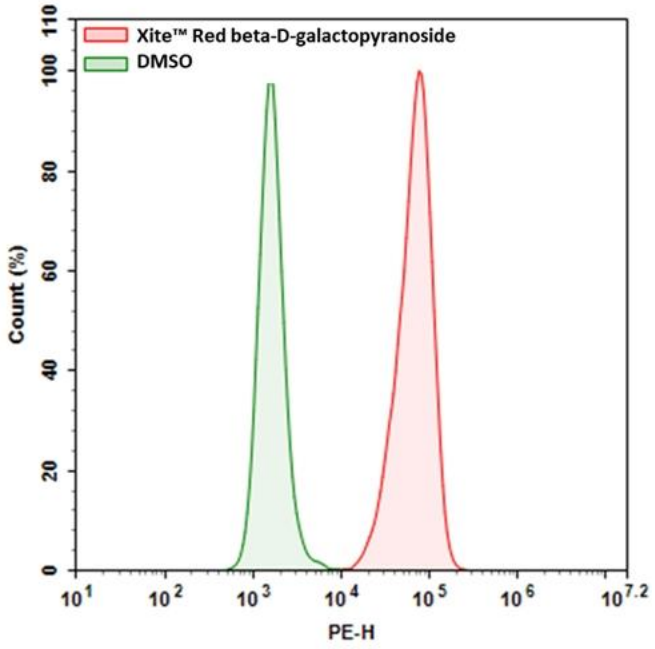

Figure 1. Expression of β-gal was measured with Xite™ Red beta-D-galactopyranoside. 9L-LacZ cells (cells that overexpressed β-gal) were incubated with Xite™ Red beta-D-galactopyranoside for 30 mins at 37 °C. The

signal was acquired with PE channel using a NovoCyte Flow Cytometer (ACEA Biosciences).

DISCLAIMER

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