PRODUCT INFORMATION SHEET

Xite™ Green beta-D-galactopyranoside

Catalog number: 14030
Unit size: 1 mg

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

OVERVIEW

Xite™ Green beta-D-galactopyranoside is a fluorogenic substrate for beta-galactosidase (β-gal). Compared to the existing beta-galactosidase substrates (e.g., the commonly used FDG), it has much better cell permeability. Xite™ Green beta-D-galactopyranoside readily enters cells where it gets cleaved by β-gal, producing Xite™ Green, a strongly fluorescent product. The strongly fluorescent Xite™ Green is well retained in cells, making it easy to be detected with a flow cytometer and fluorescence microscope. Xite™ Green beta-D-galactopyranoside provides a simple and sensitive tool to detect beta-galactosidase activity. Xite™ Green 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.

AT A GLANCE

Protocol summary

1. Treat samples as desired
2. Prepare and add Xite™ Green 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) or using fluorescence microscopy with FITC filter set

KEY PARAMETERS

Flow cytometer

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

Fluorescence microscope

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

PREPARATION OF WORKING SOLUTION

Xite™ Green beta-D-galactopyranoside working solution
Prepare 1-20 µM of Xite™ Green beta-D-galactopyranoside working solution in buffer of your choice. **Note:** Xite™ Green beta-D-galactopyranoside working solution should be used promptly. **Note** : The concentration of the Xite™ Green 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 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 Xite™ Green 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 530/30 nm filter (FITC channel) or fluorescence microscope with FITC filter set.

EXAMPLE DATA ANALYSIS AND FIGURES

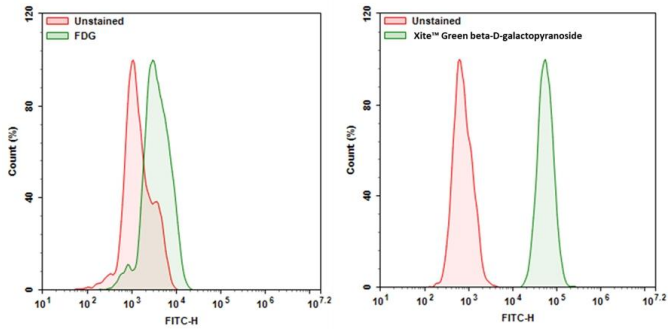

Figure 1. Expression of β-gal was measured with Xite™ Green beta-D-galactopyranoside. 9L-LacZ cells (cells that overexpressed β-gal) were incubated with Xite™ Green beta-D-galactopyranoside or FDG for 30 mins at 37 °C. The signal was acquired with FITC channel using a NovoCyte Flow Cytometer (ACEA Biosciences).

DISCLAIMER

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