β-Galactosidase Colorimetric Assay

Cat. No. 8068
50 Tests in 35mm plate

Introduction

β-Galactosidase, an important reporter gene marker encoded by lacZ, is commonly used for monitoring transfection efficiency in cultured cells and identifying expression of recombinant fusion genes. The ScienCell™ β-galactosidase Colorimetric Assay provides an easy-to-use method to detect β-galactosidase by staining cells with 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) at pH 7.4.

Kit Components

Cat. No.	# of vials	Reagent	Quantity	Storage
8068a	1	Staining Solution A	1 ml	4°C
8068b	1	Staining Solution B	1 ml	4°C
8068c	1	Staining Solution C	0.2 ml	4°C
8068d	1	Staining Solution D	100 ml	4°C
8068e	1	X-gal Solution	5 ml	-20°C
8068f	1	100× Fixing Solution	1 ml	4°C

Quality Control

Human umbilical vessel endothelial cells (HUVECs) are transfected with Promega® ρSV-bata-galactosidase control vector. β-Galactosidase Colorimetric Assay is applied to assay the gene expression 24 hours post transfection, as shown in Figure 1.

Procedures

A. Preparation of reagents

1. Preparation of working fixing solution: Prepare working fixing solution by diluting 100× Fixing Solution stock 1:100 in PBS.
2. Preparation of working staining solution: Prepare fresh working staining solution based on the number of samples to be assessed. For each sample in 35 mm plate, prepare the following mixture:

	100 µl of X-gal Solution
	20 µl of Staining Solution A
	20 µl of Staining Solution B
	4 µl of Staining Solution C
+	1856 µl of Staining Solution D
	2000 µl of working staining solution

B. Staining protocol

1. Remove culture medium from cells and rinse cells twice with PBS.
2. Fix cells by incubating with 2 ml of working fixing solution for 3-5 minutes at room temperature.

3. Aspirate fixing solution and rinse the fixed cells three times with PBS.
4. Add 2 ml of working staining solution to the cells and incubate overnight at 37°C, the blue color should develop in β-galactosidase expressing cells.*
5. Remove working staining solution and rinse cells twice with PBS, store cells in PBS at 4°C until examination under light microscope.

* Crystal deposition, which comes from unreacted X-gal, may be observed after incubation of cells with working staining solution. It can be minimized by pre-filtering the working staining solution with a 0.2 µm filter.

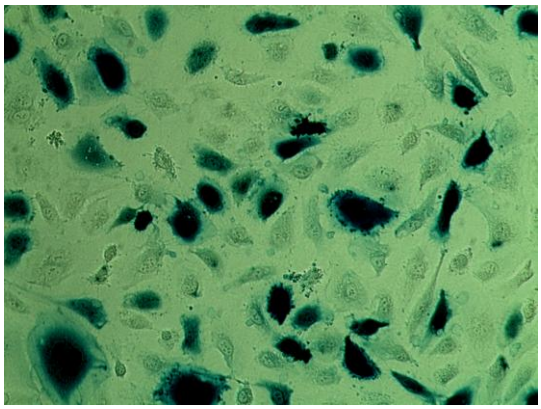

Figure 1. HUVECs transfected with Promega® ρSV-bata-galactosidase control vector.