X-Gal Staining™

Assay Kit

Cat. No.	Content	Qty	Storage
A10300K (50 assays in 60 mm dishes)	Fixing Buffer	125 ml	4°C
	10X PBS	75 ml	4°C
	Staining Buffer	125 ml	4°C
	25X X-Gal Stock (5-bromo-3-indoyl-β-D-galactopyranoside)	4 X 1 ml	-20°C

Shipping & Storage	Shipped on blue ice. The X-Gal Stock solution should be stored at -20°C. All other components should be stored at 4°C. Stable for 6 months when stored properly.

RELATED PRODUCTS	Catalog #
GenePORTER™ Transfection Reagent, 75 reactions	T201007
GenePORTER™ Transfection Reagent, 150 reactions	T201015
GenePORTER™ Transfection Reagent, 750 reactions	T201075
GenePORTER™ 2 Transfection Reagent, 75 reactions	T202007
GenePORTER™ 2 Transfection Reagent, 150 reactions	T202015
GenePORTER™ 2 Transfection Reagent, 750 reactions	T202075
gWiz™ β-galactosidase Expression Vector, 25 μg	P010200
Enhanced β-galactosidase Assay Kit (CPRG)	A10100K
β-Galactosidase Staining Kit (ONPG)	A10200K

INTRODUCTION *LacZ* is a commonly used reporter gene in transfection experiments because the gene product, β-galactosidase (β-gal), is very stable and resistant to proteolytic degradation and easily assayed. This assay kit provides all the required reagents, and offers a rapid and simple method to determine the percentage of cells transfected with *LacZ* expressing plasmids, such as Genlantis' gWiz β-gal vector. β-gal catalyzes the hydrolysis of β-galactosides (i.e. X-Gal) and cells transfected with β-gal expressing plasmid appear blue following fixation and incubation with X-Gal substrate. Blue cells can be visualized by microscopy.

USAGE:

- Transfect cells with a plasmid expressing *LacZ* gene.
- Fix the cells with formaldehyde-glutaraldehyde buffer.
- Stain the cells with X-Gal staining solution.
- Observe the cells with blue stain under a microscope.
- Calculate the percentage of stained cells in the total population versus non-transfected cells

EXPERIMENTAL PROTOCOL

A. Buffer Preparation

1. Dilute 10X PBS to 1X with distilled deionized water before use. 1X PBS may be stored at 4°C or room temperature for future use.
2. Dilute 25X X-Gal stock to 1X with Staining Buffer. Discard unused 1X X-Gal.

B. Assay Protocol

Use the following table for recommended buffer volumes to use depending on the type and size of your tissue culture plate or dish:

Type of culture dish	Fixing Buffer (μl/well)	Staining Buffer (μl/well)	1X PBS Washing Buffer (μl/well/wash)
Chambered slide	500	500	1000
24-well plate	250	250	500
12-well plate	500	500	1000
6-well plate	1000	1000	2000
60 mm dish	2500	2500	3000
100 mm dish	5000	5000	8000

1. Aspirate the medium 24-72 hours after transfection from the culture dish.
2. Wash the cells 1 time with 1X PBS.
3. Add Fixing Buffer to the dish and incubate for 10-15 minutes at room temperature.

   CAUTION: Fixing Buffer contains chemicals that are corrosive, carcinogenic, and toxic. Handle Buffer carefully (see Materials Safety Data Sheet for further details) by wearing gloves, goggles, lab coats, and protective gear.
4. Remove the fixing solution from the dish and gently wash the cells 2 times with 1X PBS.
5. Add freshly prepared 1X X-Gal staining solution to the dish. Incubate the cells between 1-18 hours at 37°C in a humidified incubator. Adjust the incubation time according to the transfection efficiency.
6. Remove the X-Gal staining solution and wash the cells 1 time with 1X PBS.
7. Add 1X PBS to the dish. Examine the dish under a light microscope; count the stained and unstained cells in randomly selected fields. Calculate the percentage of stained cells in the total population.
8. To store the plates for weeks or months, fix each well with 1ml of 10% formalin in PBS (not supplied) for 10 minutes at room temperature. Rinse with 1X PBS and store in 1X PBS or 70% glycerol solution (not supplied) at 4°C.

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