Gene Therapy Research Reagents

X-Gal Staining Assay Kit

Product Summary

Cat. No: A10300K

Description: Ready-to-use assay system for determining the transfection efficiency based on in situ β-galactosidase staining.

Components:

Component	Quantity	Storage
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

Sufficient reagents are provided to perform 50 assays of 60 mm dishes.

Comments: Suitable for use in gene delivery studies. Optimized β-gal expression vector (gWIZ™ β-gal vector) and transfection reagent (GenePORTER™) from Gene Therapy Systems are sold separately.

INTRODUCTION

LacZ is a commonly used reporter gene in transfection experiments because the gene product, β-galactosidase, 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 plasmid, such as Gene Therapy Systems' gWiz β-gal vector. β-galactosidase catalyzes the hydrolysis of β-galactosides (i.e. X-Gal) and consequently, cells transfected with β-galactosidase 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. Non-transfected cells or cells transfected with a blank plasmid should be used as control to determine the level of background activity caused by endogenous β-galactosidase.

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PROTOCOLS

Dilute 10X PBS to 1X with distilled deionized water before use. Unused 1X PBS may be stored at 4C or room temperature for future use.

Dilute 25X X-Gal stock to 1X with Staining Buffer. Discard unused 1X X-Gal.

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 poisonous and must be handled carefully (see Materials Safety Data Sheet for further details). Wear gloves, goggles, lab coats and other protective gear when handling the Fixing Buffer. Some products are harmful if inhaled, swallowed or absorbed through the skin.

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

Solution volumes recommended for various culture dishes are listed in the following table.

Type of culture dish	**Fixing Buffer (ml/well)**	**Staining Buffer (ml/well)**	**1X PBS Washing Buffer (ml/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

RELATED PRODUCTS

Product	**Catalog #**
GenePORTER™ transfection reagent	
75 transfections	T201007
150 transfections	T201015
750 transfections	T201075
gWIZ™ β-gal expression vector	
25 µg	P010200
Enhanced β-galactosidase assay kit (CPRG)	A10100K
β-galactosidase assay kit (ONPG)	A10200K

Please contact us for a complete list of pGeneGrip™ plasmid products bearing different labels and various encoded reporter genes.

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