CELL APPLICATIONS, INC.

β-Galactosidase Assay Kit: X-Gal Stain

Cat. No. GA10-300K 50 assays (60 mm dishes)

COMPONENTS

Component	Quantity	Storage (°C)
Fixing Buffer	125 ml	4
10x PBS	75 ml	4
Staining Buffer	125 ml	4
25x X-Gal Stock (5-bromo-3-indoyl -β-D-galactopyranoside)	4x 1 ml	-20

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 β-galactosidase Assay Kit provides all the required reagents for a rapid and simple method to determine the percentage of cells containing *LacZ*-xpressing plasmids. 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.
- For use with tissue sections, an increased incubation time with X-Gal Staining Solution may be necessary.
- Observe the cells with blue stain under a microscope.
- Calculate the percentage of stained cells in the total population versus non-transfected cells.

PROTOCOL

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 to create X-Gal Staining Solution.

Assay Protocol:

Use the following table to determine recommended buffer volumes based on the type and size of the tissue culture dish:

Tissue Culture Dish	Fixing Buffer (µl/well)	Staining Buffer (µl/well)	1x PBS Washing Buffer (µl/well/wash)
Chambered slide	500	500	1000
24-well	250	250	500
12-well	500	500	1000
6-well	1000	1000	2000
60 mm	2500	2500	3000
100 mm	5000	5000	8000

1. 24-72 hours post-*LacZ* DNA transfection, aspirate the medium from the culture dish.

2. Wash the cells one 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 Buffer 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 to 18 hours at 37°C in a humidified incubator. Adjust the incubation time according to transfection efficiency.

6. Remove the X-Gal Staining Solution and wash the cells one 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 1 ml 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.