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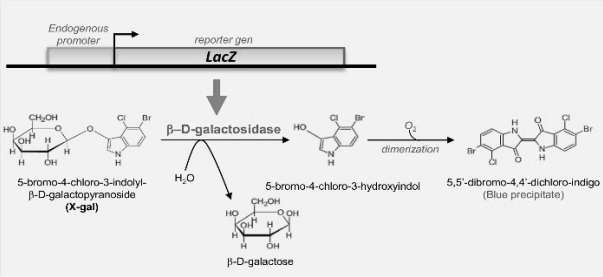
Technical Datasheet

EZdetect™ β-Gal Staining Kit

Product Code: CCK064-1KT

1. Introduction

The β-galactosidase Staining Kit provides an easy-to-use and efficient method to determine the transfection efficiency and expression of LacZ gene. Plasmid containing LacZ gene is commonly used reporter gene in transfection experiments because the gene product β-galactosidase, is very stable and resistant to proteolytic degradation and easily assayed. β-galactosidase catalyzes the hydrolysis of X-Gal, which produces blue color in cell expressing the transfected gene.



2. About the Assay

The β-Gal Staining Kit provides reagents required to determine the percentage of cells transfected with a plasmid expressing lacZ. β-galactosidase catalyzes the hydrolysis of lactose. X-Gal, produces a blue color that can be visualized under a microscope.

Component		Quantity	Storage
Part A	Staining solution A	1x3mL	-20^{0}C
Part B	Staining solution B	1x3mL	-20^{0}C
Part C	Staining solution C	1x3mL	-20^{0}C
Part D	X-Gal powder	1x300mg	-20^{0}C
Part E	Dimethyl formamide	1x15mL	RT
Part F	Fixative solution	1x30mL	-20^{0}C
Part G	Washing solution 10X	1x60mL	RT

3. Materials required but not provided in the kit

- 37^{0}C incubator
- Light microscope
- Cells or tissue samples expressing LacZ

4. Preparation of Reagents

- 1X Fixing solution: Prepare a 1X Fixing solution by diluting the Part F provided as 10X stock. Add 1 part of Part F (Fixative solution) and 9 part of PBS. Store the diluted solution at -20^{0}C temperature for up to six months.
- Dissolve 60mg X-Gal powder (Part D) in 1.5mL of DMF (Part E) for each tube.
- Dilute Part G (PBS 10X) with cell Culture grade water by adding 1 part of Part G and 9 part of cell culture grade water.
- Cell staining working solution: Prepare fresh cell staining working solution based on the number of samples. The chart below is suggested for samples in 35 mm plate, and may be modified accordingly to suit other culture plate size.

Reagent	1 plate (35mm)	5 plates (35mm)	10 plates (35mm)
Staining solution A	20µL	100µL	200µL
Staining solution B	20 µL	100µL	200µL
Staining solution C	20 µL	100µL	200µL
X-Gal solution	50 µL	250µL	500µL
Part G	1.89mL	9.45mL	18.9mL
Total	2mL	10mL	20mL

5. Staining Protocol

1. Aspirate the cell culture medium from gene transfected or infected cells from the culture dish.
2. Wash the cells twice with 3mL 1X PBS and aspirate the final wash.
3. Add 2mL of 1X fixing solution (Diluted Part F) to 35mmculture dish. Incubate at room temperature for 5 mins.
4. Remove the fixing solution and wash the fixed cells three times with 3mL of 1X PBS washing solution.
5. Aspirate the final wash, and completely cover cells by adding 2mL of freshly prepared cell staining working solution.
6. Incubate the cell at 37ºC protected from light for 1hr to overnight (This will depend on the cell lines used).
7. Remove the cell staining working solution, then wash the stained cells twice with 3mL of 1X PBS and store cells in 1X PBS. For Long term storage, overly the cells with 20% glycerol solution made in 1X PBS (Diluted Part G).
8. Count the blue stained cells using the light microscope. To determine transfection or infection efficiency, calculate the ratio of blue stained cells to total cells.

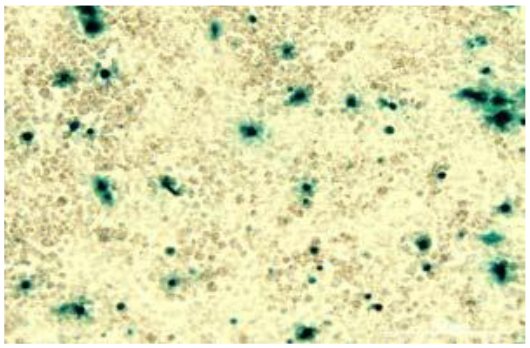


Figure showing the X-Gal staining of CHO cells transfected with expression vector plasmid containing LacZ reporter gene.

6. Storage and shelf life

- Store 10X DPBS (Part G) and 1X DPBS (Diluted Part G) at room temperature.
- Store other components at -20ºC.
- Use before expiry date mentioned on the product label.

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