

**ab102534**

# **Beta Galactosidase Staining Kit (LacZ)**

## **Instructions for Use**

For the rapid, sensitive, and accurate measurement of beta Galactosidase in various samples

This product is for research use only and is not intended for diagnostic use.

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## 1. Overview

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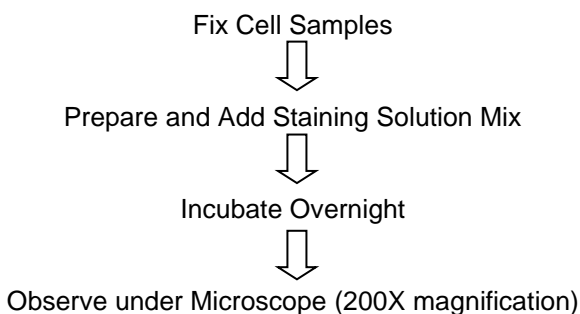
The LacZ gene from *E. coli* is one of the most commonly used reporter genes for testing the efficiency of expression vector mediated gene transfer and for studying the regulation of promoters of genes.

The LacZ gene encodes the enzyme beta-galactosidase, which is very stable, resistant to proteolytic degradation, can utilize a variety of substrates and can be easily assayed in situ. Abcam's Beta Galactosidase Staining Kit (LacZ) utilizes X-Gal as the substrate.

This kit has been optimized for transfected cells. If you want to detect senescence, we recommend using Beta Galactosidase Staining Kit (Senescence) (ab65351).

## 2. Protocol Summary

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### 3. Components and Storage

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All components in this kit are shipped on blue ice and are suitable for storage at -20°C, unless reconstituted. Upon receipt, immediately store kit at -20°C in the dark. Individual components may be stored at alternative temperatures as show in the table below. Kit has a storage time of 1 year from receipt and all components supplied are stable for 1 year.

#### A. Kit Components

Item	Quantity	Storage Temperature
Fixative Solution III	125 mL	-20°C
X-Gal	1 vial	-20°C
Staining Solution II	125 mL	+4°C
100X Staining Supplement	1.5 mL	-20°C

- For long-term storage of the stained plates, remove the Staining Solution II and overlay the cells with 70 % glycerol. Store at +4°C.

X-GAL SOLUTION: Dissolve 20 mg X-Gal in 1 ml DMSO or DMF (N-N-dimethylformamide, not provided) to prepare a 20X stock solution.

Excess X-Gal solution can be stored at -20°C (protect from light) for one month. Always use polypropylene container or glass to make and store X-Gal. Do not use polystyrene.

Staining Solution II AND 100X Staining Supplement: If precipitation occurs, simply warm up the solution to solublize the precipitates.

## **B. Additional Materials Required**

- 1X PBS Solution
- N-N-dimethylformamide
- Microcentrifuge
- Pipettes and pipette tips
- Microscope
- 12-well plate
- Orbital shaker

**Note:** The following protocol is designed for each well in a 12-well culture plate. For using large plates, increase the volume proportionally (e.g., for 6-well plate, double the volume).

## 4. Assay Protocol

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1. Prepare 1X PBS Solution (not provided). Prepare 3 ml per well. Remove culture medium and wash cells once with 1 ml of 1X PBS.
2. Fix the cells with 0.5 ml of Fixative Solution III for 10-15 min at room temperature.
3. While the cells are in the Fixative Solution, prepare the Staining Solution Mix. Using polypropylene plastic tube only. Prepare enough solution for the number of wells to be stained. For each well, prepare the following mixture:

Staining Solution II	470 $\mu$ l
100X Staining Supplement	5 $\mu$ l
20 mg/ml X-Gal in DMF	25 $\mu$ l

4. Wash the cells two times with 1 ml of 1X PBS.
5. Add 0.5 ml of the Staining Solution Mix to each well. Cover the plate. Incubate plate at 37°C (1 hour – overnight incubation)

**NOTE:** CO<sub>2</sub> levels found in general 37°C incubators will lower the pH of the Staining Solution II and affecting the color development. We suggest putting the plate inside a Ziplock® re-sealable bag to avoid any effect from the CO<sub>2</sub>.

6. Observe cells under a microscope for development of blue color (200X total magnification).



### **Technical Support**

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