

ab102534

beta Galactosidase Detection Kit

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.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

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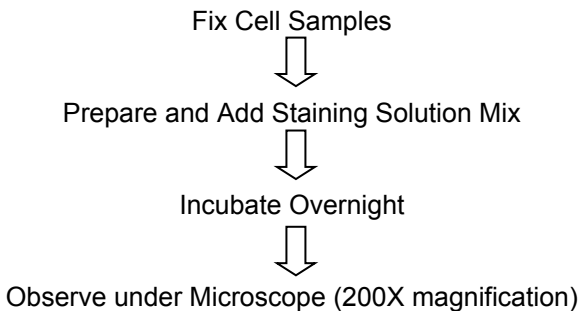
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1. Overview

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 Detection Kit utilizes X-gal as the substrate.

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
Fixative Solution III/Fixative Solution (1X)	125 mL
X-Gal/X-Gal (150 mg, Lyophilized)	1 vial
Staining Solution II/Staining Solution (1X)	125 mL
100X Staining Supplement/Staining Supplement (100X)	1.5 mL

* Store kit at -20°C, protect from light.

- Store reconstituted X-gal at -20°C.
- Store Fixative Solution III/Fixative Solution (1x) at -20°C.
- All components supplied are stable for 1 year.
- For long-term storage of the stained plates, remove the Staining Solution II/Staining Solution 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/STAINING SOLUTION AND 100X Staining Supplement/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

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/Fixative Solution 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/Staining Solution	470 μ l
100X Staining Supplement/Staining Supplement	5 μ l
20 mg/ml X-gal in DMF	25 μ 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: CO₂ levels found in general 37°C incubators will lower the pH of the Staining Solution II/staining solution and affecting the color development. We suggest putting the plate inside a Ziplock® re-sealable bag to avoid any effect from the CO₂.

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

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