β-Galactosidase Staining Kit

(Catalog #K802-250; 250 assays; Store kit at 4˚C)

I. Introduction:

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 β-galactosidase, which is very stable, resistant to proteolytic degradation, can utilize a variety of substrates and can be easily assayed in situ. The β-Galactosidase staining kit utilizes X-gal as the substrate.

II. Kit Contents:

Component	K802-250	Cap Code	Part Number
Fixative Solution (1X)	125 ml	NM	K802-250-1
X-Gal (150 mg, lyophilized)	1 vial	Green	K802-250-2
Staining Solution (1X)	125 ml	WM	K802-250-3
Staining Supplement (100X)	1.5 ml	Red	K802-250-4

III. Beta-Galactosidase Staining Protocol:

A. General Consideration & Reagent Preparations:

- The following protocol is designed for each well in a 12-well culture plate. For using large plate, increase the volume proportionally (e.g., For 6-well plate, double the volume).
- Prepare 1X PBS Solution (not provided). Prepare 3 ml per well.
- Prepare 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 and Staining Supplement: If precipitation occurs, simply warming up the solution to solublize the precipitates.

B. Staining Protocol:

1. Remove culture medium and wash cells once with 1 ml of 1X PBS.
2. Fix the cells with 0.5 ml of Fixative Solution for 10 - 15 min at room temperature.
3. While the cells 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:

   470 µl of Staining Solution
   5 µl of Staining Supplement
   25 µl of 20 mg/ml X-gal in DMF

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 overnight at 37˚C.
6. Observe cells under a microscope for development of blue color (200X total magnification).
7. For long-term storage of the stained plates, remove the Staining Solution and overlay the cells with 70 % glycerol. Store at 4˚C.

IV. Storage and Stability:

Store kit at 4˚C or –20˚C, protected from light. Store reconstituted X-gal in –20˚C. All components supplied are stable for 1 year.

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