β-Galactosidase Reporter Assay Kit

Introduction

β-Galactosidase Reporter Assay Kit is a kit for the detection of the β-galactosidase reporter gene using colorless ONPG as substrates. β-galactosidase is a commonly used reporter molecule that can be used to transfect cells with the luciferase reporter and serve as an internal control for the luciferase reporter, thereby eliminating experimental errors due to differences in transfection efficiency. The substrate provided in this kit produces a yellow product catalyzed by β-galactosidase, and the activity of β-galactosidase can be determined by measuring the absorbance at 420 nm. The lysis buffer provided in this kit is suitable for the lysis of animal cells, plant cells or tissue samples.

Components and Storage

Components	K2183-200 T
Lysis Buffer	100 mL
Assay Reagent	10 mL
Stop Buffer	35 mL
Store the kit at -20°C, stable for at least 6 months. Assay Reagent should be stored at -20°C away from light.	

Protocol

1. **Reagent preparation:** Lysis Buffer, Assay Reagent, and Stop Buffer are melted in advance and warmed to room temperature. If Stop Buffer contains refractory crystals, put it in a 37°C water bath to make it dissolved.
2. **Cell lysis:** Refer to the table below to lyse cells by adding an appropriate amount of Lysis Buffer per well on ice. The Lysis Buffer can be pipetted several times to make full contact with the cells. After the cells were fully lysed, centrifugation at 10,000-14,000 g at 4ºC for 3-5 min, and the supernatant was taken as a sample for subsequent testing. Lysed samples can be tested immediately or frozen at -80ºC. Cryopreserved samples need to be warmed to room temperature prior to testing.

	96-well plate	48-well plate	24-well plate	12-well plate	6-well plate
Lysis Buffer	100 μL	150 μL	200 μL	300 μL	500 μL

3. **Reaction:** Prepare a new 96-well plate, add the appropriate amount of sample (5-50 μL recommended), and then replenish to a volume of 50 μL with Lysis Buffer. Due to the large differences in different cell systems and

the different dosages required for different samples, it is recommended to do a pre-experiment to determine the amount of samples before the first test. Then add 50 μL of Assay Reagent, mix well, and incubate at 37ºC for 30 minutes or until the sample wells appear light yellow. For longer incubation times, the 96-well plate can be sealed with parafilm or plastic wrap to minimize liquid evaporation.

***Note:** After approximately 3 h of incubation, the reaction reaches a plateau, and the absorbance does not increase significantly if the incubation is longer.

4. **Stop the reaction:** Add 150 μL of Stop Buffer per well to stop the reaction, mix well, and take care to avoid bubbles as much as possible. When first added, it may be cloudy, but when it is mixed well, it becomes a clear liquid.
5. **Detection:** Measure absorbance at 420 nm using a microplate reader. If a 420 nm filter is not available, a 410-430 nm filter can be used as well.

Note

1. For your safety and health, please wear lab coats and gloves during the experiment.
2. For research use only. Not to be used in clinical diagnostic or clinical trials.

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