



Bio-UltraBio™ Bright two- step Firefly Luciferase Reporter Gene Assay Kit (Lyophilized)

B1506973

Storage: -20°C. Protect from light.

Introduction

Genetic reporters are used commonly in cell biology to study gene expression and other cellular events coupled to gene expression, such as receptor activity, intracellular signal transduction, mRNA processing, protein folding and protein:protein interactions. Firefly luciferase is one of the most commonly used reporter genes in gene expression. It is an extremely sensitive, fast-responding and easy-to-use reporter gene. The chemiluminescence reaction it catalyzes is one of the most sensitive analytical tools for measuring gene expression.

Firefly luciferase is a protein with a molecular weight of approximately 61 kDa. In the presence of ATP, magnesium ions and oxygen, it can catalyze the oxidation of luciferin into oxyluciferin. During the oxidation process of luciferin, bioluminescence with a wavelength of approximately 560 nm is emitted, and this fluorescence can be measured by a chemiluminescence analyzer.

Bio-UltraBio™ Bright two-step Firefly Luciferase Reporter Gene Assay Kit (Lyophilized) is a highly sensitive, glow-type quantitative detection kit, particularly suitable for samples with low expression levels of firefly luciferase. This kit is an alternative packaging version of the [B1373068Z] Bio-UltraBio™ Bright two-step Firefly Luciferase Reporter Gene Assay Kit (Ready-to-use), with comparable detection signal intensity. Both kits exhibit a signal half-life of approximately 30 minutes. The detection reagent in the [B1373068Z] Bio-UltraBio™ Bright two-step Firefly Luciferase Reporter Gene Assay Kit (Ready-to-use) is supplied as a ready-to-use liquid, offering the advantage of direct use without reconstitution. However, it requires storage at -80°C, and prolonged storage at -20°C may gradually reduce its detection performance. In contrast, this lyophilized powder product remains highly stable when stored at -20°C. For initial use, the firefly luciferase substrate must be reconstituted with the provided firefly luciferase detection buffer. The assay buffer included in the kit can be stored at room temperature ($\leq 25^{\circ}\text{C}$), thereby saving time required for temperature equilibration.

Kit Contents

B1506973	Component	100 T	10×100 T	Storage conditions	Quantity Per Test
B1506973A	5×Luciferase Assay Lysis Buffer	10 mL	10×10mL	-20°C.	100 µL
B1506973B	Firefly luciferase detection buffer	10 mL	10×10mL	-20°C.	100 µL
B1506973C	Firefly luciferase substrate	1 vial	10 vials	-20°C. Store in the dark.	100 µL (After reconstitution)

Instructions for Use

1. Cell lysis

- 1) Adherent cells: Aspirate the culture medium completely.
Suspension cells: Centrifuge to collect the cells and discard the supernatant.
- 2) Add an appropriate volume of 1× Lysis Buffer (prepared by diluting Component A 4:1 with sterile water) according to the culture vessel, as referenced in the table below:

Culture Vessel	96-well plate	48-well plate	24-well plate	12-well plate	6-well plate
Lysis Buffer Volume (µl/well)	100	150	200	300	500

Note: If luciferase expression level is low, consider using less lysis buffer (e.g., as low as 100 µl/well for a 6-well plate).

- 3) Lyse the cells for 10 minutes at room temperature, either on a microplate shaker or by standing.
- 4) For 96-well plates: Lysates can be assayed directly or frozen for later analysis. Thaw frozen samples completely and equilibrate to room temperature before assaying.
- 5) For other vessel types: Centrifuge lysates at 10,000-15,000 × g for 3-5 minutes. Use the supernatant for the assay.

2. Reagent preparation

Completely pour the Firefly Luciferase Detection Buffer into the vial containing the Firefly Luciferase Substrate. Invert the vial several times to fully dissolve the substrate.

*Note: If the reconstituted Firefly Luciferase Detection Reagent is not fully used in a single experiment, it is recommended to aliquot it on ice immediately after reconstitution. The remaining aliquots should be stored at -20°C or -80°C.

3. Instrument Settings

Turn on the luminometer or a multifunctional microplate reader with chemiluminescence detection capability. Set instrument parameters according to the manufacturer's instructions and experimental requirements.

4. Sample Loading

Load 20-100 µl of cell lysate (or supernatant for centrifuged samples) for measurement (100

μl is recommended if sample volume permits; use a consistent volume across all samples in the same experiment). Use an equal volume of lysis buffer as a blank control.

5. Measurement

Add 100 μl of the Firefly Luciferase Detection Reagent to each sample. Mix thoroughly and measure the luminescent signal immediately.

Precautions

1. The 5× Luciferase Assay Lysis Buffer can be stored short-term at 4°C, is stable for up to one year at -20°C, and can be stored long-term at -80°C.
2. For initial use, the firefly luciferase substrate must be reconstituted with the provided Firefly Luciferase Assay Buffer.
3. Repeated freeze-thaw cycles of the reconstituted Firefly Luciferase Substrate Detection Reagent should be minimized. It is recommended to aliquot the solution upon initial reconstitution and store it protected from light at -20°C or -80°C.
4. Avoid prolonged exposure of the reconstituted Firefly Luciferase Substrate Detection Reagent to room temperature.
5. The enzymatic reaction is temperature-sensitive. Both the cell culture plate and the detection reagent must be equilibrated to room temperature prior to assay initiation.
6. Detector Selection: Any instrument capable of measuring chemiluminescence is suitable for use with this kit. However, for identical samples, the background signal and measured values may vary between different detectors. Furthermore, readings obtained from different instruments for the same sample are not directly comparable.
7. To prevent well-to-well crosstalk, the use of opaque white or black cell culture plates is recommended.