Bio-UltraBio™ Steady one-step Firefly Luciferase Reporter Gene Assay Kit (Lyophilized)

B1511295

Storage -20°C. Protect from light.

Shipping Shipped with ice packs. Please store under the specified storage conditions immediately upon receipt.

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™ Steady one-step Firefly Luciferase Reporter Gene Assay Kit (Lyophilized) is a one-step assay kit for directly measuring the intracellular firefly luciferase activity through chemiluminescence without the need for cell washing or collection. It features high sensitivity and ultra-high signal stability. This kit is a different packaging version of [S1375171] Bio-UltraBio™ Steady one-step Firefly Luciferase Reporter Gene Assay Kit (Ready-to-use), and detection signal intensities of the two are comparable. The signal half-life is approximately 5 hours. The detection reagent in the [S1375171] Bio-UltraBio™ Steady one-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.

Kit Contents

B1511295	Component	100 T	10×100 T	Storage conditions	Quantity Per Test
B1511295A	Firefly luciferase detection buffer	10 mL	10×10mL	-20°C.	100 μL
B1511295B	Firefly luciferase substrate	1 vial	10×1 vials	-20°C. Store in the dark.	100 μL (After reconstitution)

Instructions for Use

1. 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.

2. Cell lysis

1) Adherent cells: Do not aspirate the cell culture medium; directly add the detection reagent of the same volume as the medium.
   Suspension cells: The same as adherent cells.
2) Recommended usage volume:

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

3) Lyse the cells for 5 minutes at room temperature, either on a microplate shaker or by standing.

3. Sample Addition

Samples in 96-well plates may proceed directly to the subsequent assay. For other sample types, pipette 200 μl of the mixed solution (culture medium + detection reagent) per well into the assay plate.

4. Detection

Initiate the chemiluminescence detector or a multifunctional microplate reader equipped with chemiluminescence functionality. Set parameters according to the instrument manual and experimental requirements, then proceed to measure the signal values.

Precautions

1. For initial use, the firefly luciferase substrate must be reconstituted with the provided Firefly Luciferase Assay Buffer.

2. 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.
3. Avoid prolonged exposure of the reconstituted Firefly Luciferase Substrate Detection Reagent to room temperature.
4. 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.
5. 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.
6. To prevent well-to-well crosstalk, the use of opaque white or black cell culture plates is recommended.