Single Firefly Luciferase Reporter Gene Assay Kit

Product description

Firefly luciferase is a protein with a molecular weight of about 61 kD that catalyzes the oxidation of the substrate luciferin to oxyluciferin in the presence of ATP, oxygen and magnesium ions. During the oxidation of Luciferin, a bioluminescence with a wavelength of about 560 nm is emitted, which can be determined by a luminometer or liquid flash analyzer. With luciferin and luciferase as bioluminescent systems, gene expression can be detected very sensitively and efficiently. Generally, the transcriptional regulatory element of the gene of interest or the 5' promoter region is cloned upstream of luciferase, or the 3'-UTR region is cloned downstream of luciferase, etc., to construct a reporter gene plasmid, transfect the cells and treat the cells with appropriate drugs, and finally determine the luciferase activity of the cell lysate and judge the transcriptional regulation effect of drug treatment on the target gene by the level of luciferase activity (Fig 1).

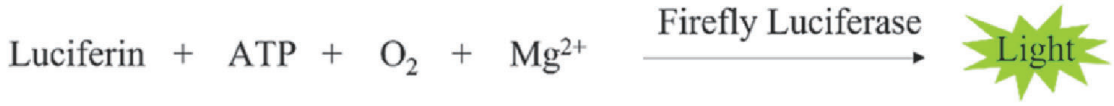

Fig. 1. Schematic diagram for the detection of firefly fluorophore enzymes

The Single Firefly Luciferase Reporter Gene Assay Kit is a highly sensitive, highly signal-stable one-step assay for direct chemiluminescence determination of intracellular Firefly luciferase activity without washing or collecting cells.

The firefly luciferase detection reagent provided in this kit can be added to the cell culture plate in the same volume as the culture medium for 5 minutes to react, and chemiluminescence detection can be performed. This kit is flexible and convenient to use, has high detection sensitivity, stable luminescence signal, wide linear range of measured samples, and better performance than major domestic similar products (Fig 2). In addition, this product can also be used for the detection of lysing and collecting preserved cell samples.

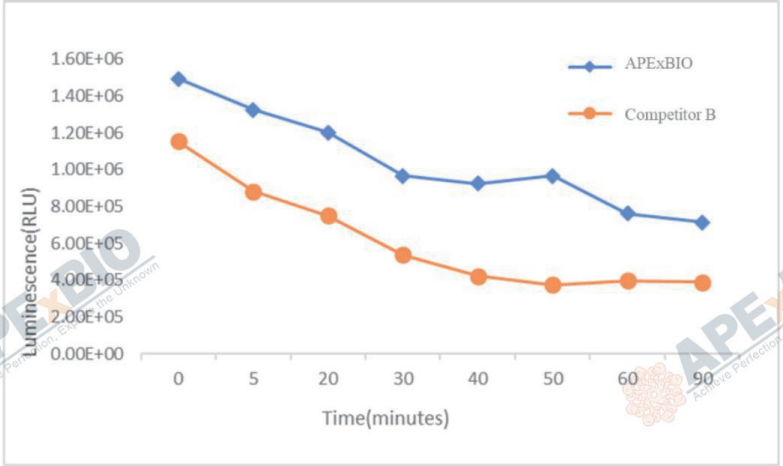

Fig. 2 Comparison of firefly luciferase reporter gene detection experiments. The Single Firefly Luciferase Reporter Gene Assay Kit (K2236) improved the luminescence intensity by about 30% compared with that of Competitor B, and the stability of chemiluminescence signal was significantly better than that of Competitor B.

Components and storage conditions

Components	K2236-100 T	K2236-1000 T
Single Firefly Luciferase Assay Reagent	10 mL	100 mL
Store at -80ºC for one year or at -20ºC for 3 months away from light.		

Experimental manipulation

Cells are detected directly after treatment

1. **Prepare the cells to be tested**

   Seed 100 μL of cells per well using a 96-well plate suitable for chemiluminescence detection (e.g., 25 μL of cells per well using a 384-well plate, depending on the type of 384-well plate) and set up cell-free culture wells as negative controls to culture and transfect cells according to conventional methods of cell culture and cell transfection. If necessary, drug-treated cells can be added.

2. **Prepare the assay**

   thawed and frozen Single Firefly Luciferase Assay Reagent, prepare the assay at an amount of 100 μL per well in a 96-well plate (25 μL per well in a 384-well plate) and equilibrate to room temperature.

3. **Luciferase test**

   3.1 Remove the cell culture plate and equilibrate at room temperature for 10 min (usually should not exceed 30 min).

   3.2 Add 100 μL of the assay prepared in the previous step to each well of the 96-well plate (25 μL per well of the 384-well plate).

3.3 Incubate for 5 min at room temperature (approximately 25 °C) to stabilize the luminescence signal.

3.4 Chemiluminescence detection using a versatile microplate reader with detection chemiluminescence capabilities. Please set the corresponding parameters according to the instrument requirements, the detection time of each well is generally 0.25-1 second or longer, and the specific needs to be adjusted appropriately according to the detection sensitivity of the instrument.

Cell lysate assay

Note: Consider only if the cell volume is large, such as when the cells are cultured in a dish or 6-well plate.

1. Cell preparation

   - For adherent cells: after aspiration of the cell culture medium, add an appropriate amount of firefly luciferase reporter gene cell lysate according to the table below;
   - For suspension cells: After centrifugation to remove the supernatant, add an appropriate amount of firefly luciferase reporter cell lysate according to the table below.

Utensil type	96-well plates	48-well plates	24-well plates	12-well plates	6-well plates
Reporter gene cell lysate (μL/well)	100	150	200	300	500

Note: If luciferase expression levels are low, try using less lysate, for example, a minimum of 100 μl per well for a 6-well plate.

2. After adequate lysis, centrifuge at 10,000-15,000 g for 3-5 min and take the supernatant for the assay.

Note: Luciferase can be measured immediately after cell lysis, or frozen and determined later. Cryopreserved samples need to be thawed and measured at room temperature.

3. Take 20 μL of the sample, add 100 μL of Single Firefly Luciferase Assay Reagen that has been thawed and equilibrated to room temperature, and mix appropriately.

4. Incubate for 5 min at room temperature (approximately 25°C) to stabilize the luminescence signal.

   Note: If the requirements for data stability are not too high, you can ignore this step and perform chemiluminescence detection immediately after mixing.

5. Chemiluminescence detection using a versatile microplate reader with detection chemiluminescence capabilities. Please set the corresponding parameters according to the instrument requirements, the detection time of each well is generally 0.25-1 second or longer, and the specific needs to be adjusted appropriately according to the detection sensitivity of the instrument.

Notes

1. The detection effect of this product when stored at -20°C will gradually decrease, and its luminescence effect will be reduced by about 50% after half a year of storage. Therefore, if stored at -20°C, this product is recommended for use within 3-6 months.
2. Since luciferase activity is temperature-sensitive, cells and detection reagents need to be measured at room temperature before reaction. The detection reagent can be used after melting and mixing well at room temperature or in a water bath not exceeding 25°C.
3. Although repeated freeze-thawing of this reagent 5 times has no significant effect on its detection effect, in order to ensure the stability of this product and achieve good use effect, the method of storing it in the dark after appropriate aliquoting after the first thaw can be taken to avoid repeated freeze-thaw and long-term exposure to room temperature. Repeated freeze-thaw processes may cause a small amount of precipitation in the detection reagent, which should be balanced to room temperature and dissolved as much as possible. If there is still residual insoluble matter, it can be used after centrifugation removal, and the test will not affect the subsequent detection effect.
4. Use a white or black 96-well plate or a 384-well plate suitable for cell culture. If a normally transparent 96-well plate or a 384-well plate is used, adjacent wells can interfere with each other.
5. Higher solvent levels of the drug to be tested may interfere with the luciferase reaction and thus affect the chemiluminescence signal. Solvent-containing cell culture medium control wells can be set up to exclude solvent interference. After testing, DMSO content within 2% of the final reaction system will not affect the reaction.
6. In order to avoid errors caused by differences in cell transfection efficiency, if necessary, the reporter plasmid of sea kidney luciferase can be transfected as an internal reference, which can be detected by the Dual Luciferase Assay System Dual Luciferase Assay System Dual Luciferase Assay Kit (K1136).
7. This product is for scientific use only.

APExBIO Technology
www.apexbt.com
7505 Fannin street, Suite 410, Houston, TX 77054.
Tel: +1-832-696-8203 | Fax: +1-832-641-3177 | Email: info@apexbt.com