Biotium, Inc.
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Firefly Luciferase Assay Kit

Catalog Number: 30003-1 (150 assays)
30003-2 (1000 assays)

Contact Information

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Description

Firefly luciferase is widely used as a reporter for studying gene regulation and function, and for pharmaceutical screening[1, 2]. It is a very sensitive genetic reporter due to the lack of any endogenous activity in mammalian cells or tissues[3, 4]. The *Firefly* luciferase is a 62,000 Dalton protein, which is active as a monomer and does not require subsequent processing for its activity. The enzyme catalyzes ATP-dependent D-luciferin oxidation by oxygen into oxyluciferin with emission of light centered on 560nm (Figure 1). As with many enzymes, *Firefly* luciferase follows Michaelis-Menten kinetics and, as a result, maximum light output is not achieved until the substrate and co-factors are present in large excess. When assayed under these conditions, light emitted from the reaction is directly proportional to the number of luciferase enzyme molecules. This *Firefly* luciferase assay kit is designed for detection and quantification of the *Firefly* luciferase reporter enzyme from cultured cells in a simple, efficient, and linear fashion (Figure 2).

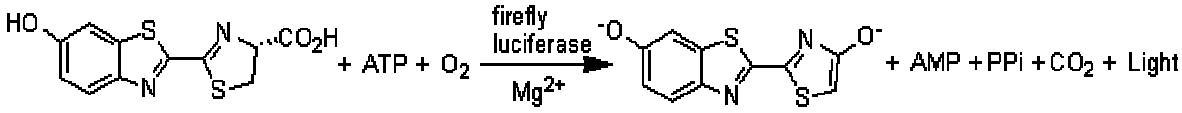

Figure 1.Bioluminescent reaction catalyzed by *Firefly* luciferase.

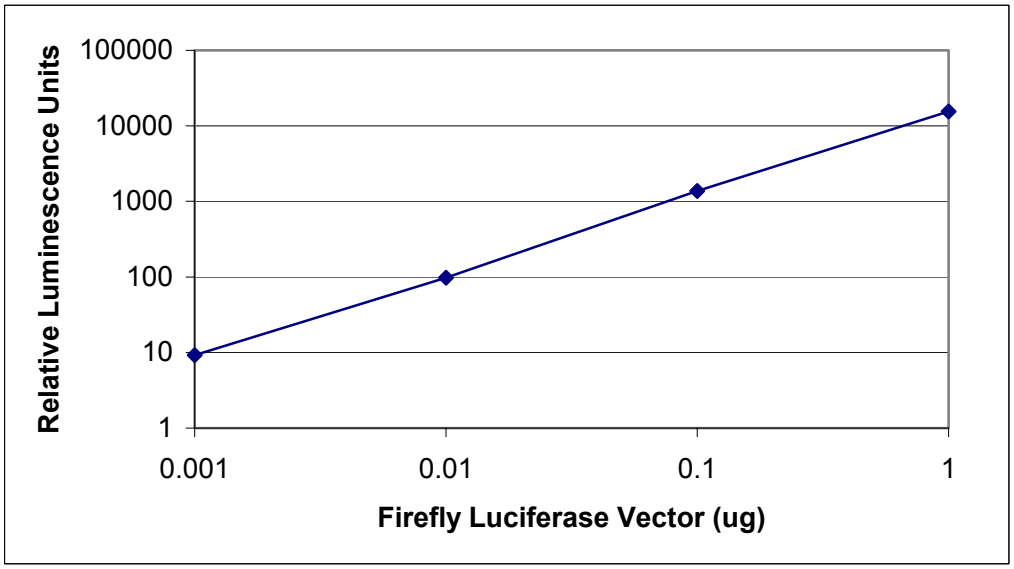

Figure 2. Dose response curve of transfected *Firefly* Luciferase gene. PC3 cells were transfected with 0.001μg, 0.01μg, 0.1μg, and 1μg pFL-CMV vector encoding *Firefly* luciferase gene by Fugene 6 (Roche) in 6-well cell culture dishes. pGL2 Basic vector (Promega) was used as a control and for normalizing total DNA vector level to 1μg per transfection. Twenty-four hours after transfection, cells were harvested using 500μL lysis buffer contained in Biotium's *Firefly* Luciferase Assay Kit. To assay luciferase activity, 20μL of lysate from each sample was then added to 100μL of assay buffer also in Biotium's *Firefly* Luciferase Assay Kit. Luminescence was measured on a luminometer (Turner Designs). Light emission was integrated over 10 seconds without initial pre-read delay.

Product Components

***Firefly* Luciferase Assay Kit, 30003-1 (150 assays)**
3 vials (1mg each) of D-Luciferin
15mL 5X *Firefly* Luciferase Assay Lysis Buffer
15mL *Firefly* Luciferase Assay Buffer

***Firefly* Luciferase Assay Kit, 30003-2 (1000 assays)**
2 vials (10mg each) of D-Luciferin
30mL 5X *Firefly* Luciferase Assay Lysis Buffer
100mL *Firefly* Luciferase Assay Buffer

Storage Conditions

Store *Firefly* Luciferase Assay Kit at –20°C. Kit components are stable for three months at –20°C and up to six months at –70°C. *Firefly* luciferase assay solution (Assay Buffer + Substrate) should be prepared fresh for each use. Avoid repeated freeze-thaw cycles. Aliquot *Firefly* Luciferase Assay Buffer for storage if necessary.

Preparation of Cell Lysates

A. Preparation of *Firefly* Luciferase Lysis Buffer

Firefly Luciferase Lysis Buffer 1X working solution is prepared by adding 1 volume of 5X *Firefly* Luciferase Lysis Buffer to 4 volumes of de-ionized water and mixing well. The 1X Lysis Buffer may be stored at 4°C for up to one month. Store the 5X *Firefly* Luciferase Assay Lysis Buffer at –20°C.

B. Lysis of Cells Cultured in Multiwell Plates

1. Remove growth medium from cultured cells and gently add a sufficient volume of phosphate buffered saline (PBS) to wash the surface of the culture vessel. Add *Firefly* Luciferase Assay Lysis Buffer to each well using the volume recommended below for each type of culture plate:

6 well culture plate: 500µL per well
12 well culture plate: 250µL per well
24 well culture plate:100µL per well
48 well culture plate: 65µL per well
96 well culture plate: 20µL per well

2. Place the culture plates on a rocking platform or orbital shaker with gentle rocking/shaking to ensure complete and even coverage of the cell monolayer with 1X *Firefly* Luciferase Lysis Buffer. Rock the culture plates at room temperature for 15 minutes.

Note: Cultures that are overgrown are often more resistant to complete lysis and typically require an increased volume of *Firefly* Luciferase Lysis Buffer and/or an extended treatment period to ensure complete lysis. Lifting cells from the plate will facilitate the process of cell lysis.

3. Transfer the lysate to a tube or vial and place in 4°C for further assay. Although it is not necessary, the lysate can be cleared by centrifugation for 30 seconds at top speed in a refrigerated microcentrifuge and transferred into a new tube.

Firefly Luciferase Assay

A. Preparation of *Firefly* Luciferase Assay Solution

1. Thaw a bottle of *Firefly* Luciferase Assay Buffer and pipette a desired volume (5mL or 50mL) from the bottle into a new container.

2. Dissolve the supplied D-luciferin with the above assay buffer at 0.2mg/mL concentration in a clean container. 5 mL assay solution is prepared from each 1 mg D-luciferin vial. Similarly, 50 mL assay solution is prepared from each 10 mg D-luciferin vial. *Firefly* Luciferase Assay Solution should be prepared fresh and used within a day.

Note: D-luciferin in Assay Buffer has limited stability. If you need less than 5mL or 50mL Luciferase Assay Solution as described in step 2, you may dissolve D-Luciferin in DI water as 10X or 50X stock solution and store it at -20°C or below for repeated use. The D-luciferin stock solution should be stable for at least one month, depending on the frequency of free-thaw cycle. A desired volume of the final Assay Solution can be prepared by diluting the stock solution with the supplied Assay Buffer to 0.2mg/mL D-luciferin.

B. Standard Protocol

For manual luminometer:

1. Set up luminometer with appropriate parameters (delay time, integration time, sensitivity, etc.).

2. Add 100µL of *Firefly* Luciferase Assay Solution to the luminometer tube.

3. Add 20µL of cell lysate. Mix quickly by flicking the tube with a finger for thorough mixing.

3. Place tube in luminometer and initiate measurement. Luminescence is normally integrated over 10 seconds without delay. Other integration times may also be used.

4. If the luminometer is not connected to a printer or computer, record the *Firefly* luciferase activity measurement.

5. Discard the reaction tube, and proceed to the next *Firefly* Luciferase Assay.

For luminometer with injector:

1. Format the luminometer so that the injector dispenses 100µL. Prime the injector with *Firefly* Luciferase Assay Solution.

2. For each reaction, carefully add 20µL of cell lysate to an individual luminometer tube or to the wells of a multiwell plate.

3. Place the samples in a luminometer.

4. Initiate measurement. This action will cause *Firefly* Luciferase Assay Solution to be injected into the reaction vessel and the measurement to be subsequently taken. Luminescence is normally integrated over 10 seconds without delay. Other integration times may also be used.

5. Record the *Firefly* luciferase activity measurement.

6. If using a single tube luminometer, discard the reaction tube, and proceed to the next *Firefly* Luciferase Assay reaction. If using a plate luminometer, the luminometer will automatically begin injecting *Firefly* Luciferase Assay Solution into the next well indicated on the luminometer plate.

References

1. Alam, J. and J.L. Cook. 1990. Reporter genes: Application to the study of mammalian gene transcription. Anal. Biochem. 188:245-254.
2. Bronstein, I., et al. 1994. Chemiluminescent and bioluminescent reporter gene assays. Anal. Biochem. 219:169-181.
3. Gould, S.J. and S. Subramani. 1988. *Firefly* luciferase as a tool in molecular and cell biology. Anal. Biochem. 175:5-13.
4. Brasier, A.R., et al. 1989. Optimized use of the *Firefly* luciferase assay as a reporter gene in mammalian cell lines. BioTechniques. 7:1116-1122.