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Revised: December 13, 2013

Product Information

Firefly Luciferase Assay Kit

Catalog Number: 30003-T, 30003-1, 30003-2

Kit Contents

Component	30003-T 50 assays	30003-1 150 assays	30003-2 1000 assays
5X Firefly Luciferase Lysis Buffer	5 mL 30003B-T	15 mL #99923	2 x 15 mL #99923
Firefly Luciferase Assay Buffer	5 mL #99933	15 mL #99932	100 mL #99909
D-Luciferin	1 x 1 mg #99907	3 x 1 mg #99907	2 x 10 mg #99908

Note: Sufficient firefly lysis buffer is provided to perform the stated number of assays with cells grown in 96 – 24 well plates. For applications requiring more lysis buffer (e.g. >100 uL/well), additional 5X lysis buffer (Cat. # 99923) may be purchased separately.

Storage and Handling

Store the kit at –20°C or below. The kit is stable at –20°C for three months and at –70°C for at least six months from date of receipt. Aliquot assay buffer if necessary to avoid repeated freeze-thaw cycles. Firefly luciferase working solution (assay buffer + D-luciferin) should be prepared fresh on the day of assay.

Product 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 absence of endogenous luciferase activity in mammalian cells or tissues.[3, 4] 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 to oxyluciferin, producing light emission centered at 560 nm (Figure 1). 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 simple and efficient quantitation of firefly luciferase reporter enzyme activity from cultured cells with high sensitivity and linearity (Figure 2). This is a flash-type luminescence assay with signal half-life of about 12 minutes. Biotium also offers the Steady-Luc HTS Firefly Assay Kit (cat. no. 30028), which is a homogenous glow-type assay with signal half-life of 3-5 hours.

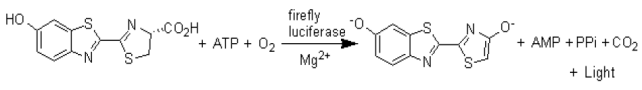

Figure 1.Bioluminescent reaction catalyzed by firefly luciferase.

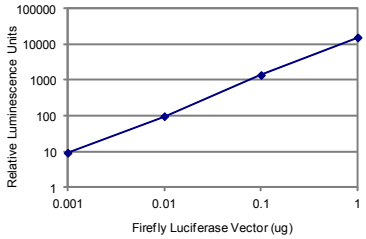

Figure 2. Dose response curve of transfected firefly luciferase gene. PC3 cells were transfected with 0.001 ug, 0.01 ug, 0.1 ug, and 1 ug pFL-CMV vector encoding firefly luciferase gene by Fugene 6 (Roche) in 6-well cell culture dishes. pGL2 Basic vector (Promega) was used as a control and for normalizing total DNA vector level to 1 ug per transfection. Twenty-four hours after transfection, cells were harvested using 500 uL lysis buffer contained in Biotium's Firefly Luciferase Assay Kit. To assay luciferase activity, 20 uL of lysate from each sample was then added to 100 uL 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.

Assay Protocols

Preparation of Cell Lysates

Preparation of Firefly Luciferase Lysis Buffer

1. Prepare 1X firefly luciferase lysis buffer by adding 1 volume of 5X firefly luciferase lysis buffer to 4 volumes of dH_2O and mixing well. 1X lysis buffer may be stored at 4°C for up to one month. Store 5X firefly luciferase lysis buffer at –20°C.

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 1X firefly lysis buffer to each well using the volume recommended below for each type of culture plate:

Wells/plate	Lysis buffer/well
6 well	500 uL
12 well	250 uL
24 well	100 uL
48 well	65 uL
96 well	20 uL

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. Biotium offers mini cell scrapers (cat. no. 22003) for harvesting lysates from 96-, 24-, and 48-well plates.

3. Transfer the lysate to a tube or vial. Optional: the lysate can be cleared by centrifugation for 30 seconds at top speed in a refrigerated microcentrifuge and transferred into a new tube. Place at 4°C for until ready to assay. Store lysates at -20°C or -70°C if assay will not be performed on the same day.

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Firefly Luciferase Assay

Preparation of Firefly Luciferase Working Solution

1. Prepare an adequate volume of working solution to perform the desired number of firefly luciferase assays (100 uL working solution per assay). Thaw a bottle of firefly luciferase assay buffer and pipette a desired volume (5 mL or 50 mL) from the bottle into a clean container.
2. Dissolve the supplied D-luciferin in the firefly luciferase assay buffer from step 1 at a final concentration of 0.2 mg/mL. For kit 30003-1, dissolve one vial of D-luciferin (component 99907, 1 mg/vial) in 5 mL assay buffer. For kit 30003-2, dissolve one vial of D-luciferin (component 99908, 10 mg/vial) in 50 mL assay buffer. Firefly luciferase working solution (D-luciferin + firefly luciferase assay buffer) should be prepared fresh and used within a day.

   Note: D-luciferin in assay buffer has limited stability. If you need less than 5 mL or 50 mL luciferase working solution as described in step 2, you may dissolve D-luciferin in dH_2O as 10X or 50X stock solution and store it in aliquots 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 freeze-thaw cycles. The required volume of working solution can be prepared by diluting the stock solution in firefly luciferase assay buffer to a final concentration of 0.2 mg/mL D-luciferin.

Standard Protocol

For manual luminometer:

1. Set up luminometer with appropriate parameters (delay time, integration time, sensitivity, etc.).
2. Add 100 uL of firefly luciferase working solution to the luminometer tube.
3. Add 20 uL of cell lysate and mix quickly by vortexing or flicking the tube with a finger.
4. Place tube in luminometer and initiate measurement. Luminescence is normally integrated over 10 seconds without delay. Other integration times may also be used.
5. If the luminometer is not connected to a printer or computer, record the firefly luciferase activity measurement.
6. Discard the reaction tube, and proceed to the next firefly luciferase reaction.

For luminometer with injector:

1. Format the luminometer so that the injector dispenses 100 uL. Prime the injector with firefly luciferase working solution.
2. For each reaction, carefully add 20 uL 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 will cause firefly luciferase working 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 also may 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 reaction. If using a plate luminometer, the luminometer will automatically begin injecting firefly luciferase working 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.

Related Products

Catalog number	Product
30004	Renilla Luciferase Assay Kit
30005	Firefly & Renilla Dual Luciferase Assay Kit
30028	Steady-Luc Firefly HTS Assay Kit
30020	ATP-Glo Bioluminometric Cell Viability Assay
99923	5X Firefly Luciferase Lysis Buffer, 15 mL
22003	Mini Cell Scrapers, pack of 200

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