Revised: February 21, 2017

Product Information

Firefly Luciferase Assay Kit 2.0

Catalog Number: 30085-T, 30085-1, 30085-2

Kit Contents

Component	30085-T 50 assays	30085-1 150 assays	30085-2 1000 assays
5X Firefly Luciferase Lysis Buffer	5 mL 30003B-T	15 mL #99923	2 x 15 mL #99923
Firefly Luciferase Assay Buffer 2.0	5 mL 99815-5mL	15 mL 99815-15mL	100 mL 99815-100mL
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. Firefly Luciferase Assay Buffer 2.0 is stable at –20°C for three months and at –80°C for at least six months from date of receipt. The other kit components are stable at -20°C for at least six months from date of receipt. Kit components and D-luciferin stock solutions in water are stable to at least 5 freeze-thaw cycles.

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 that requires signal to be measured immediately after adding working solution to samples. The luminescence signal decays over the course of about 10 minutes of reaction time, although signal half-life may vary depending on luciferase expression levels. 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 (see related products).

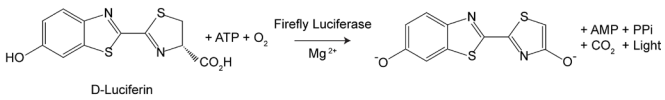

Figure 1.Bioluminescent reaction catalyzed by firefly luciferase.

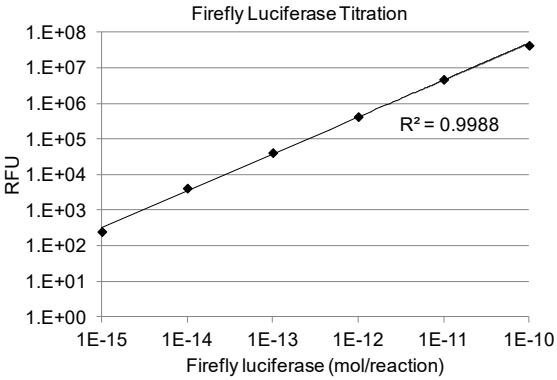

Figure 2. Titration of recombinant firefly luciferase in the Firefly Luciferase Assay 2.0. Quantilum® Luciferase (Promega) was serially diluted in 1X Firefly Lysis Buffer with 1 mg/mL BSA and measured in the assay. Luminescence was measured on a Promega Glomax® 20/20 single tube luminometer with integration time of 1 second. Background from reagents without enzyme added was subtracted from luminescence values.

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Assay Protocols

Preparation of Cell Lysates

Preparation of Firefly Luciferase Lysis Buffer

1. Prepare 1X 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

2. Remove the growth medium from the cultured cells and gently wash the cells once with a sufficient volume of phosphate buffered saline (PBS) to cover the surface of the culture vessel. Remove the PBS and add 1X passive lysis buffer using the volume recommended below for each type of well:

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

3. 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 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.

4. 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 -80°C if assay will not be performed on the same day.

Preparation of Firefly Working Solution

1. Thaw Firefly Luciferase Assay Buffer 2.0 at room temperature.
2. Prepare 10 mg/mL D-luciferin stock solution. For component 99907 (1 mg), add 100 uL water to the vial and mix. For component 99908 (10 mg), add 1 mL water to the vial and mix. The stock solution can be stored for at least 6 months at -20°C or below, and is stable to up to 5 freeze/thaw cycles.
3. Prepare enough firefly working solution to perform the desired number of assays (100 uL working solution per assay). Add D-luciferin (10 mg/mL) to assay buffer at a ratio of 1:50. For example, add 20 uL D-luciferin stock solution to 1 mL firefly assay buffer.

   Note: For best results, working solutions (assay buffer with substrate) should be prepared fresh before each use, and used within 3 hours of preparation. Firefly working solution activity decreases ~10% after 3 hours and ~25% after 5 hours at room temperature.

Firefly Luciferase Assay

The protocol below is for manual assay using a single-tube luminometer. If your luminometer is equipped with automatic injectors, they may be used to dispense working solution into each luminometer tube or well of a multiwell plate according to the instructions for your instrument.

1. Set up luminometer with parameters recommended for your instrument. We routinely use integration time of 1 second.
2. Add 20 uL of cell lysate into a reaction tube that is compatible with your luminometer.
3. Add 100 uL of firefly working solution to the reaction tube and mix by pipetting or vortexing.
4. Immediately place tube in luminometer and record the firefly luminescence measurement.

Determination of Assay Background

The expression of a luciferase reporter is quantified by the luminescence produced above background levels. In most cases, background created by the reagent in the absence of luciferase is very low compared to signal with luciferase. However, when measuring low levels of luciferase activity, it is important to subtract the background signal from untransfected cells or cells transfected with a negative control vector from measurements of luciferase activity.

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
99923	5X Firefly Lysis Buffer, 15 mL
22003	Mini Cell Scrapers, pack of 200
30081	Firefly & *Renilla* Luciferase Single Tube Assay Kit
30075	Firefly Luciferase Assay Kit (Lyophilized)
30082	*Renilla* Luciferase Assay Kit 2.0
30028	Steady-Luc™ Firefly HTS Assay Kit
30028L	Steady-Luc™ Firefly HTS Assay Kit, Lyophilized
30020	ATP-Glo™ Bioluminometric Cell Viability Assay

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