Rev. 0

ScienCell™ Research Laboratories

Luciferase Assay Kit (LAK)

Catalog #9048

100 tests

Product Description

The Luciferase Assay Kit (LAK), is designed for rapid and sequential quantification of Firefly and Renilla luciferase activities from a single sample. This dual-reporter setup enables accurate normalization of experimental variation—such as differences in transfection efficiency or cell viability—by using Renilla luciferase as an internal control. Firefly luciferase produces a bright yellow-green luminescent signal (~560 nm) via ATP-dependent oxidation of its specific substrate. This reaction is highly efficient, and both the enzyme and substrate exhibit low toxicity, making Firefly luciferase a widely used reporter in biological assays. In contrast, Renilla luciferase produces a distinct blue light (~480 nm) via its specific substrate oxidation in an ATP-independent reaction (see Figure 1). Both luciferases exhibit relatively short half-lives compared to fluorescent proteins, allowing real-time analysis of dynamic cellular responses. Due to their non-overlapping emission spectra and separate substrates, both enzymes can be independently measured from the same lysate with minimal signal interference [1-4].

The assay workflow measures Firefly luminescence first, then introduces a reagent that suppresses Firefly signal and simultaneously activates Renilla luminescence within the same well. This sequential measurement minimizes variability and streamlines the experimental procedure by eliminating the need for separate samples. Normalizing Firefly activity to Renilla luminescence enhances the precision of detecting specific biological changes. The system is widely used to quantify Firefly and Renilla luciferase activities in cultured mammalian cells, supporting high-throughput screening based on luminescence assays, as well as functional studies of gene expression, transcriptional regulation, and cellular signaling pathways.

Kit Components

Cat. #	# of vials	Product Name	Quantity	Storage
9048a	1	Firefly Assay Buffer	5 mL	4°C
9048b	1	Firefly Substrate, Lyophilized	3 mg	-20°C (protect from light)
9048c	1	Renilla Assay Buffer	5 ml	4°C
9048d	1	Renilla Substrate (100X)	50 µl	-80°C (protect from light)
9048e	1	1x Cell Lysis Buffer	5ml	-20°C

Materials Supplied by User (Not provided)

- Mammalian cells expressing Firefly luciferase under the experimental promoter and Renilla luciferase under a control promoter.
- Appropriate cell culture medium.
- White or opaque 96-well plate for luminescence reading.
- Luminometer or luminescence reader.

Product Use
LAKFR is for research use only and is not approved for human or animal use, or application in clinical or *in vitro* diagnostic procedures.

Quality Control
Assay performance was validated using HEK293T cells co-transfected with TCF/LEF Dual Reporter Vector (TDRV, Cat. #9038) following by treatment (LiCl 10mM) in a 96 well plate. Serial dilutions of lysates prepared in Lysis Buffer were tested with the Dual Luciferase reagents following this protocol. Both Firefly and Renilla luminescence signals showed a linear correlation across dilutions, confirming dynamic range, sensitivity, and assay robustness (see Figure 2).

Shipping and Storage
Kit components are shipped on dry ice. Upon receipt, store the product according to the conditions specified in the provided storage table.

References
[1] De Wet, J.R. et al. (1987). Firefly luciferase gene: structure and expression in mammalian cells. *Molecular and Cellular Biology*, 7(2), 725–737.
[2] Lorenz, W.W., et al. (1991). Isolation and expression of a cDNA encoding Renilla reniformis luciferase. *PNAS*, 88(10), 4438–4442.
[3] Badr, C.E. & Tannous, B.A. (2011). Bioluminescence imaging: progress and applications. *Trends in Biotechnology*, 29(12), 624-633.
[4] Hall, M.P., Unch, J., et al. (2012). Engineered luciferase reporter from a deep-sea shrimp utilizing a novel imidazopyrazinone substrate. *ACS Chemical Biology*, 7(11), 1848–1857.

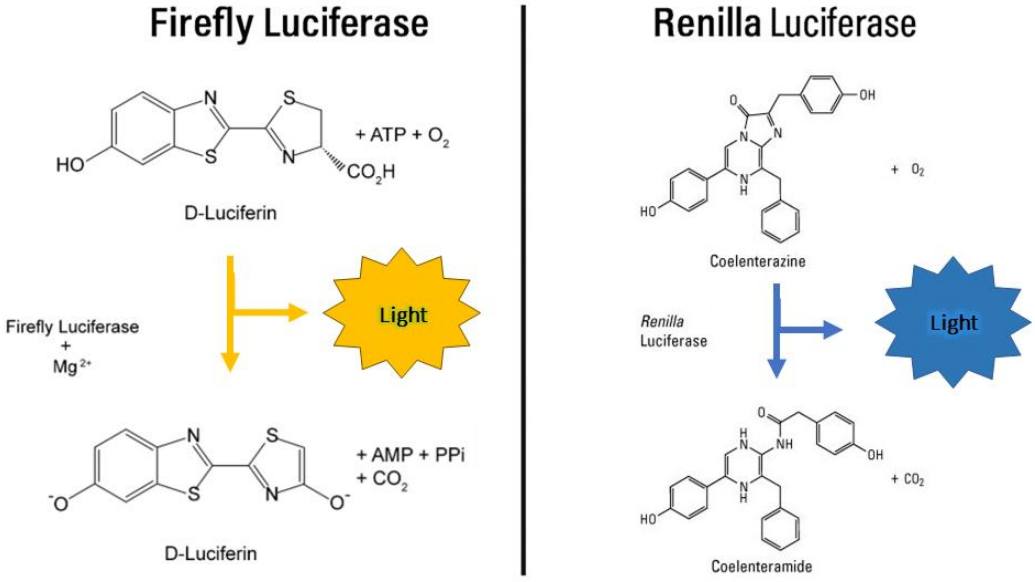

Figure 1. Chemical reactions of Firefly and Renilla luciferases with their respective substrates. Firefly luciferase oxidizes D-luciferin in an ATP-dependent reaction, producing yellow-green light (~560 nm), while Renilla luciferase oxidizes coelenterazine in an ATP-independent reaction, producing blue light (~480 nm). Both reactions occur in the same sample for dual-reporter quantification.

Procedure

1. **Reagent Preparation**
   - **A. Firefly Substrate Work Solution:** Reconstitute lyophilized Firefly Substrate in 5 mL Firefly Assay Buffer. Mix gently by inversion. Aliquot and store at –20°C protected from light. Stable for up to 2 weeks. Avoid repeated freeze-thaw cycles. Working Solution must be at room temperature before assay use. Dispense and equilibrate to room temperature only the reagent volumes needed for the number of plates being used.
   - **B. Renilla Substrate Work Solution:** Dilute 50 µL of 100× Renilla Substrate into 5 mL Renilla Assay Buffer. Mix gently. Aliquot and store at –80°C protected from light. Stable for up to 2 weeks. Thaw on ice before use. Avoid repeated freeze-thaw cycles. Briefly centrifuge Renilla Substrate (100×) before use. Working Solution must be at room temperature before assay use. Dispense and equilibrate to room temperature only the reagent volumes needed for the number of plates being used.

Note: Firefly and Renilla substrates are provided separately for sequential measurement and to minimize signal interference. Assay performance depends on transfection efficiency, reporter expression, and cell type; optimize accordingly.

2. **Sample Preparation: Cell Seeding and Transfection**
   - **A.** Seed approximately 30,000 cells per well in a white or opaque 96-well plate and incubate overnight at 37°C in a 5% CO_2 incubator. If using a different plate format, adjust the cell number accordingly. Be sure to plate enough wells to perform the experiment in triplicate and to include appropriate controls, such as non-transfected cells to assess background signal.
   - **B.** Transfect cells with Firefly and Renilla luciferase plasmids using standard protocols in the culture format of your choice.
   - **C.** Incubate cells for 16-72 hours at 37°C in 5% CO2 in a cell culture incubator.
   - **D.** Proceed with the individual experimental protocol for cell treatment.

3. **Sample Preparation: Cell Lysate**
   - **A.** Remove medium and gently wash cells twice with 50-100µL/well of Dulbecco's Phosphate-Buffered Saline (Cat#0303). Do not disturb the cell monolayer during the DPBS rinse
   - **B.** Add an appropriate volume of 1x Cell Lysis Buffer to each well, depending on well size (See table for reference), waiting for 10 minutes. Check for complete cell lysis using a light microscope. if lysis is incomplete, waiting for 10 additional minutes.

Culture Plate Format	Recommended Volume of 1× Cell Lysis Buffer
100 mm × 20 mm dish	1-2 mL
60 mm × 15 mm dish	1 mL
35 mm × 12 mm dish	500-700 µL
6-well plate	500-700 µL per well
12-well plate	300-400 µL per well
24-well plate	150-250 µL per well
96-well plate	50-100 µL per well

Assay Procedure

Step 1: Sample Preparation

Important: Prepare fresh Working Solutions as described in the Reagent Preparation section.

A. Add 20 µL of cell lysate from your experimental samples into the wells of a white opaque 96-well plate.

B. Include control wells with lysate from non-transfected cells and wells containing lysis buffer only for background luminescence measurement.

C. When running multiple plates, include a common internal control sample in each plate to allow normalization across plates.

Step 2: Firefly Luciferase Measurement

A. Set the luminometer to detect luminescence at the appropriate wavelength for Firefly luciferase (~560 nm), if not, proceed with detection using the instrument's default luminescence mode and autogain, as Firefly luciferase does not require wavelength filtering.

B. Add 50 µL of Firefly Luciferase Assay Reagent (Firefly substrate) to each well.

C. Gently mix by pipetting up and down or briefly shaking the plate.

D. Immediately measure the luminescence using the luminometer.

E. Record the relative light units (RLU) for Firefly luciferase.

Step 3: Renilla Luciferase Measurement

A. After Firefly measurement, add 50 µL of Renilla Luciferase Assay Reagent (containing Renilla substrate) directly into the same well.

B. Mix gently as before.

C. Set the luminometer to detect Renilla luciferase emission (~480 nm), or the luminescence mod like for Firefly read.

D. Measure luminescence immediately.

E. Record the RLU for Renilla luciferase.

Step 4: Data Analysis

A. To obtain the normalized luciferase activity, subtract the background luminescence, then calculate the ratio of Firefly luminescence to Renilla luminescence from the control Renilla luciferase vector.

Note: While immediate measurement is recommended, the signal remains detectable for at least 10 minutes, gradually decaying over time.

Additional Notes

Use fresh pipette tips to avoid cross-contamination. Protect luciferase reagents from light, minimize freeze-thaw cycles, and measure promptly to prevent signal loss.

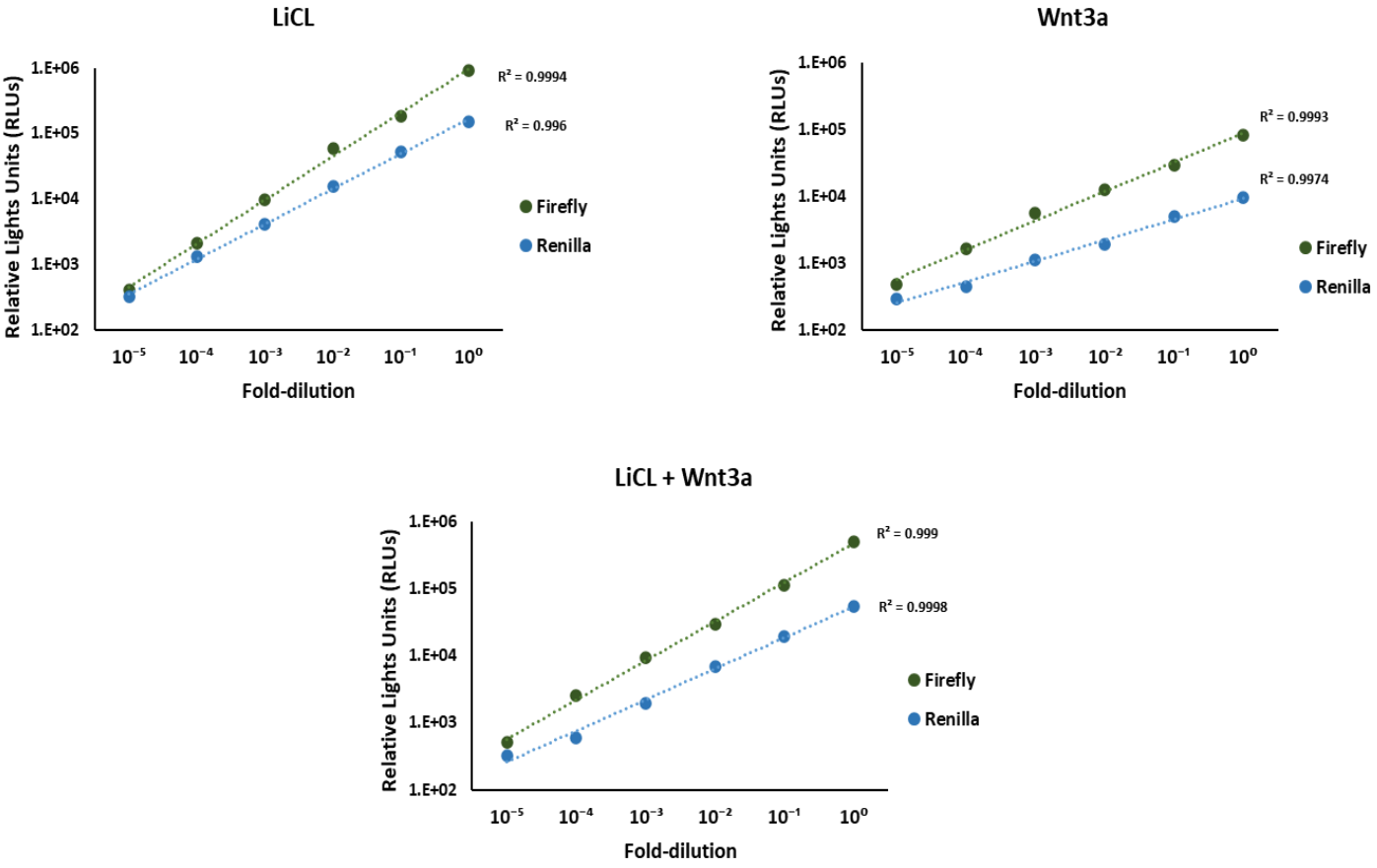

Figure 2. Linear response of Firefly reporter and Renilla control across 10^{0}–10^{-5} lysate dilutions in cells transfected with TCF/LEF Dual Reporter Vector (TDRV, Cat#9038), under stimulation with LiCl, Wnt3a, or LiCl+Wnt3a. Both enzymes show high linearity (R^2>0.995), supporting reliable Firefly/Renilla normalization.