



TECHNICAL MANUAL

Duo-Lux Luciferase Assay System

- **SKU CODES:** ASRV00017-10 / ASRV00017-100
- **SIZE:** 10ml / 100ml
- **DETECTION PRINCIPLE:** Luminescence
- **RUO:** Research-Use-Only

Duo-Lux Luciferase Assay System

Please read entire manual carefully before starting experiment.

TABLE OF CONTENTS

1. Product Description	3
2. Kit Contents & Storage	5
3. Protocol	7
5. Important Notes	11

1. Product Description

Duo-Lux Luciferase Assay System is a next-generation, high-sensitivity, and homogeneous reagent solution engineered for the rapid, concurrent quantitation of two reporter enzymes, Firefly luciferase and Renilla luciferase, from a single cell sample. Designed for simplicity, the convenient, two-step "Add-Mix-Read" protocol generates both stable luminescent signals directly from cells cultured in medium, eliminating the need for pre-conditioning, washing, or centrifugation steps.

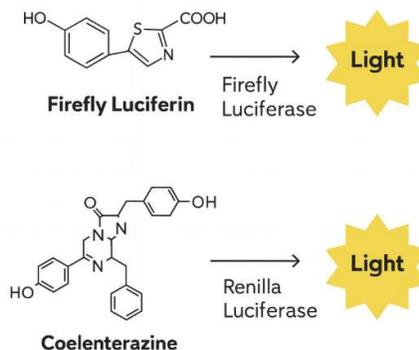
Key Features

The core strength of the **Duo-Lux System** lies in its ability to maximize data accuracy and reliability through internal normalization.

- **Primary Signal:** Firefly luciferase activity reports on the specific biological effect or stimulus under investigation.
- **Internal Reference:** Co-expressed *Renilla* luciferase serves as the **internal reference** to correct and normalize the primary results. This capability effectively eliminates the influence of common experimental variables, including fluctuations in cell number, variations in transfection efficiency, and non-specific changes in cell growth status.

Sequential Detection Mechanism

The Duo-Lux System employs high-purity firefly luciferin and coelenterazine substrates in a two-step reaction:



1. Step 1 (Firefly Read): The initial Duo-Lux Detection Reagent is added to the culture, inducing complete cell lysis and providing the substrate for the Firefly reaction. The resulting stable signal has a retention time of up to 2 hours, allowing ample time for reading.
2. Step 2 (*Renilla* Read): The Duo-Lux Stop & Lux Detection Reagent is subsequently introduced. This reagent simultaneously terminates the Firefly luminescence (with a quenching efficiency exceeding 10,000-fold) and provides the substrate for the *Renilla* reaction. This secondary stable signal can also be reliably read within 2 hours of reagent addition.

HTS performance and advantages

- **HTS Ready:** The system is optimally suited for high-throughput detection in 96- and 384-well plates, providing the speed and stability required for automated workflows.
- **Broad Compatibility:** Duo-Lux delivers broad compatibility across common mammalian cell culture systems and remains unaffected by serum concentration in the culture medium.
- **Operational Simplicity:** The Add-Mix-Read design allows reagents to be added directly to the medium, bypassing time-consuming preparation steps. The assay is compatible with any standard luminometer, and on-board injectors are not required.
- **Signal Stability:** The extended stability of both luminescent signals (up to 2 hours each) provides exceptional flexibility for continuous or batch processing of large plate quantities.
- **Exceptional Dynamic Range:** The assay allows for the analysis of both high and low reporter activity without sample dilution, offering an extremely wide dynamic range for each reporter.

A Great High-Performance Alternative

The Duo-Lux Luciferase Assay System is a great alternative to Promega Dual-Glo® Luciferase Assay System.

2. Kit Contents & Storage

Product	Code	Contents
Duo-Lux Luciferase Assay System (10ml)	ASRV00017-10	Duo-Lux Luciferase Buffer 10ml Duo-Lite Luciferase Substrate (lyophilized) 1 vial Duo-Lux Stop & Lux Buffer 10ml Duo-Lux Stop & Lux Substrate 100ul
Duo-Lux Luciferase Assay System (100ml)	ASRV00017-100	Duo-Lux Luciferase Buffer 100ml Duo-Lite Luciferase Substrate (lyophilized) 1 vial Duo-Lux Stop & Lux Buffer 100ml Duo-Lux Stop & Lux Substrate 1ml

Duo-Lux Luciferase Assay System components are stored based on their stability to facilitate convenient reconstitution. Transport should occur at temperatures less than or equal to 0°C.

Substrate Components (Temperature Sensitive)

Long-Term Storage: Store the Duo-Lux Luciferase Substrate and the Duo-Lux Stop & Lux Substrate at -30°C to -15°C. Short-Term Storage: These substrates also may be stored at 2°C to 8°C for up to 14 days. Freeze-Thaw Tolerance: The overall system remains stable after up to 10 cycles of freezing and thawing.

Buffer Components (Room Temperature Stable)

Storage: Store the Duo-Lux Luciferase Buffer and the Duo-Lux Stop & Lux Buffer below 25°C. Buffer storage at room temperature is recommended. This prevents the need for temperature equilibration when the substrates are reconstituted and the working reagents are prepared.

Working Reagents (Once Mixed)

Duo-Lux Luciferase Detection Reagent: Use immediately on the day it is prepared, or store at -70°C if not used for a long time. (Note: Storage at 2°C to 8°C provides greater

than 90% activity for 1 day, and room temperature provides greater than 80% activity for 1 day.)

Duo-Lux Stop & Lux Detection Reagent: Should be prepared on the day it is to be used

Additional Materials Required:

- Single/multi-channel pipettor
- Opaque-walled multiwell plates suitable for 3D cell culture (e.g., white or black plates)
- Microplate reader with a luminescence detection module
- 22°C water bath

3. Protocol

Reagent Preparation

1. **Thawing of Duo-Lux Buffer:** Incubate the Duo-Lux buffer at 2 to 8°C or room temperature to thaw. The product can also be incubated at 22°C in a water bath but the temperature should not exceed 25°C.
2. **Preparation of Duo-Lux Luciferase Detection Reagent:** Add the entire bottle of thawed Duo-Lux Luciferase Buffer into the Duo-Lux Luciferase Substrate. Gently invert and mix it well for 3 to 5 times until the substrate is dissolved thoroughly.
3. **Preparation of Duo-Lux Stop & Lux Detection Reagent:** Calculate the volume of reagent required for the experiment. Dilute the Duo-Lux Stop & Lux Substrate into the corresponding volume of Duo-Lux Stop & Lux Buffer at a ratio of 1:100. Gently invert and mix the solution well.
 - a. *Example:* To prepare 5 ml of Duo-Lux Stop & Lux detection reagent, add 50ul of Duo-Lux Stop & Lux Substrate into 5 ml of Duo-Lux Stop & Lux Buffer.
4. **Temperature Equilibration:** Before use, ensure that both the Duo-Lux Luciferase Buffer and Duo-Lux Stop & Lux Buffer have been equilibrated to room temperature.
 - **Frozen Reagents:** If the working Duo-Lux Detection Reagents were stored at -20°C or -70°C, they should be gently inverted and mixed well 3-5 times after thawing and before use.

Detection steps

1. Remove the cell culture plate to be tested from the incubator and equilibrate at room temperature for 30 min.
2. Firefly (Primary Reporter) Detection: Add an equal volume of Duo-Lux Luciferase Detection Reagent to the cell culture medium to be tested and then mix well.
 - a. Example 96-well: Add 75ul reagent to 75ul cell culture medium

- b. Example 384-well: Add 20ul reagent to 20ul cell culture medium
3. Measure: Incubate at RT for up to 10 minutes and detect the Firefly luminescence signal
4. Renilla Detection: Add an equal volume of Duo-Lux Stop & Lux Detection Reagent to the well, based on the original culture volume, and mix it well.
 - a. Example 96-well: Add 75ul reagent to 75ul cell culture medium
 - b. Example 384-well: Add 20ul reagent to 20ul cell culture medium
5. Measure: Incubate at RT for up to 10 minutes and detect the Renilla luminescence signal

Detection Step Note 1: The Duo-Lux Stop & Lux reagent must be added to the well within 4 hours after the Duo-Lux Luciferase reagent was added (Step 2).

Detection Step Note 2: The Renilla luminescence must be measured in the same plate order as that of the Firefly signal to maintain data integrity).

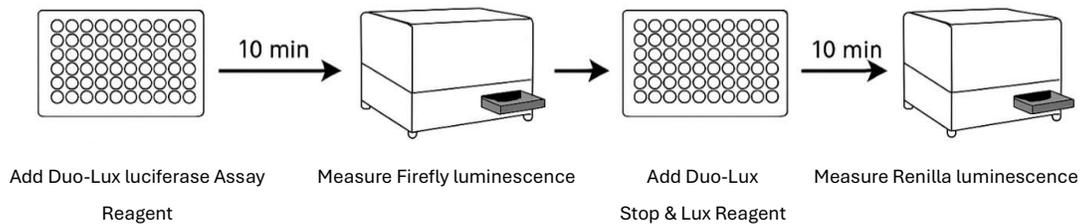


Figure 2. Short Protocol for Duo-Lux. The "Add-Mix-Read" format provides fast and simple sequential quantitation of dual reporters from a single sample. The protocol involves two main additions: First, the Duo-Lux Luciferase Assay Reagent is added directly to the cell culture, followed by an incubation and measurement of the Firefly signal. Second, the Duo-Lux Stop & Lux Reagent is added to quench the Firefly signal and initiate the *Renilla* reaction. The resulting stable *Renilla* luminescence is then measured, completing the dual-reporter reading sequence. This homogeneous method eliminates washing and pre-lysis steps, maximizing efficiency for high-throughput applications.

Additional Protocol Considerations

Mixed Reagent Handling: Cryopreservation effectively reduces the loss of activity of the Duo-Lux Luciferase Detection Reagent. Do not thaw the mixed detection reagent at a temperature higher than 25 degrees C. It is recommended to keep it in a water bath at 22 degrees C for a period of time before use to ensure it is properly equilibrated to room temperature.

Stop & Lux Preparation: Prepare only the required amount of Duo-Lux Stop & Lux Detection Reagent immediately before the experiment to ensure optimal results. This reagent should be prepared for immediate use.

Temperature Control: The intensity of luminescence and the rate of decay are directly dependent on the reaction rate of the luciferase enzymes. The optimal temperature for the activity of both luciferases is approximately room temperature (20 degrees C to 25 degrees C). Therefore, it is crucial to fully equilibrate the reagent and the culture plate to room temperature before assay initiation.

Buffer Storage: It is recommended to preserve the Duo-Lux Luciferase Buffer and Duo-Lux Stop & Lux Buffer at room temperature. If the temperature of any working reagent is lower than room temperature, place it in a 22 degrees C water bath to balance before use.

Batch Operation Control: For batch operations, set the same control well(s) on each multiwell plate to ensure the comparability and reliability of results between plates.

Multiwell Plate Selection: We recommend using standard opaque-walled multiwell plates (e.g., white or black flat-bottom plates) specifically designed for luminescence measurements. It is important to note that the luminescent intensity measured will differ based on the type of plate used: **White Plates:** Effectively reduce optical loss, leading to a higher signal, but may exhibit a certain degree of interference between wells (cross-talk). **Black Plates:** Effectively reduce cross-talk between wells, but result in a greater optical loss, leading to diminished signal intensity. **Alternative Plates:** Opaque-walled cell culture plates with transparent bottoms are also suitable for luminescence detection. However, assays performed in these plates will typically exhibit diminished

signal intensity and increased cross-talk between wells. Plates should be selected based on the specific requirements of the experiment.

4. Data Analysis

To ensure accuracy, the raw luminescence values obtained for both Firefly and Renilla luciferase signals must be subtracted from their corresponding background values before final calculation.

Background Subtraction

Background Measurement	Components in Well
Background Firefly	Non-transfected cells + Duo-Lux Luciferase Detection Reagent
Background Renilla	Non-transfected cells + Duo-Lux Luciferase Detection Reagent + Duo-Lux Stop & Lux Detection Reagent

Important Note: The sample volume used for background measurement must be the same as the experimental sample volume. Additionally, the background wells should contain the same culture medium/serum combination as the experimental sample.

Control Groups and Normalization

According to different experimental purposes, the following groups should be set on each culture plate:

- **Blank Control (Normalized Zero Point):** Non-transfected cells after deduction of the corresponding background signals (i.e., Background Firefly and Background Renilla). This establishes the baseline signal for the assay.
- **Experimental Group:** Transfected cells treated with an experimental compound. This yields the experimental Firefly signal and the experimental *Renilla* signal.

Final Normalized Calculation

The final result expresses the Relative Activity or Fold Change of the experimental condition compared to the untreated control group, after internal normalization by the *Renilla* standard.

Control Group Definition: The Control Group consists of transfected cells that received no treatment (vehicle only).

$$\text{Final Result} = \frac{(\text{Experimental Group Firefly Value} - \text{Background Firefly Value}) / (\text{Experimental Group Renilla Value} - \text{Background Renilla Value})}{(\text{Control Group Firefly Value} - \text{Background Firefly Value}) / (\text{Control Group Renilla Value} - \text{Background Renilla Value})}$$

5. Important Notes

1. This kit is intended for Research Use Only. Assay Genie assumes no responsibility for any issues or legal liabilities arising from the use of this kit for clinical diagnostics or any other unauthorized purposes.
2. Please read the instructions carefully before beginning the assay. Ensure that all instruments are correctly calibrated. Strict adherence to the protocol is essential for accurate results.
3. Appropriate laboratory safety precautions must be followed, including the use of lab coats and latex gloves.
4. If the concentration of the target substance falls outside the detection range, please adjust the sample by performing further dilution or concentration as needed.
5. Experimental outcomes depend on multiple factors including reagent integrity, handling technique, and laboratory conditions. While Assay Genie guarantees the quality of our kits, we are not responsible for any loss of samples during use. We advise calculating sample requirements in advance and ensuring adequate sample volume is reserved before starting the assay.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

