TECHNICAL MANUAL

Stable-Lux Luciferase Assay System

- **SKU CODES:** ASRV00014-10 / ASRV00014-10x10 /ASRV00014-100
- **SIZE:** 10ml / 10x10ml / 100ml
- **DETECTION PRINCIPLE:** Luminescence
- **RUO:** Research-Use-Only

Revised: 11/2025

Stable-Lux Luciferase Assay System

Please read entire manual carefully before starting experiment.

TABLE OF CONTENTS

1. Product Description

Stable-Lux Luciferase Assay System is a homogeneous reagent solution designed for ultra-high sensitivity and stable quantitation of firefly (*Photinus pyralis*) luciferase expression in mammalian cells. This system is optimized for batch processing of 96- and 384-well plates, delivering long-lived luminescence when added directly to cultured cells.

The homogeneous assay provides a highly stable luminescent response, making it ideal for high-throughput applications. Throughput rates of several thousand samples per hour can be achieved with high reproducibility under standard laboratory conditions.

Technology and Stability

Stable-Lux contains high-purity luciferin and an optimized reaction reagent formulation. This results in enhanced stability and greater tolerance to environmental factors compared to traditional assays.

The Steady-Lux Detection Reagent facilitates a homogeneous, single-step reaction: Upon direct addition to the cell culture medium, the mixed reagent immediately induces cell lysis, effectively releasing the intracellular luciferase enzyme. The reagent contains high-purity Luciferin and an optimized reaction composition that stabilizes the light output. This generates a strong, stable optical signal with enhanced tolerance to environmental factors.

The Steady-Lux assay is highly sensitive and reliable, exhibiting less susceptibility to variances in mixing conditions across multiwell plates compared to other extended-lifetime assays. This enables throughput rates of several thousand samples per hour to be achieved with high reproducibility under standard laboratory conditions.

Assay Advantages

- **No Sample Preprocessing:** No requirement to remove culture medium or wash cells prior to adding the assay reagent. Cells can be grown and assayed directly in the same multiwell plate.
- **Ultra-high Sensitivity:** The optimized formulation provides ultra-high sensitivity and a more stable light output and improved detection limits.
- **Convenient and Homogeneous:** To prepare the working reagent, simply mix the provided Buffer with the lyophilized Substrate. The assay is performed by adding the reagent directly to the cells in culture, waiting a short time, and measuring luminescence.
- **High Stability:** The optimized reagent chemistry ensures a stable luminescent signal, promoting reliable reading across large sample sets.

A Great High-Performance Alternative

The Stable-Lux Luciferase Assay system is a great alternative to Promega Steady-Glo® Luciferase Assays.

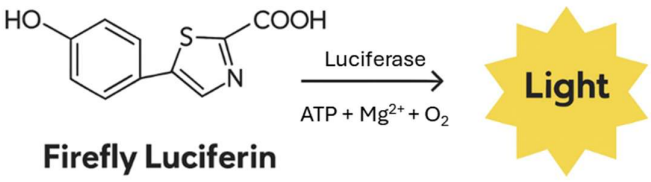

Figure 1. Schematic of Stable-Lux Detection Principle. The mono-oxygenation of luciferin is catalyzed by the thermostable luciferase in the presence of magnesium ions (Mg^{2+}), molecular oxygen (O_2), and ATP. The ATP required for this reaction is contributed directly by the viable cells following reagent-induced lysis.

2. Kit Contents & Storage

Product	Code	Contents
Stable-Lux Luciferase Assay System (10ml)	ASRV00014-10	Stable-Lux Luciferase Assay Buffer (10ml) Stable-Lux Luciferase Assay Substrate (lyophilized) 1 Vial
Stable-Lux Luciferase Assay System (10x10ml)	ASRV00014-10x10	Stable-Lux Luciferase Assay Buffer (10x10ml) Stable-Lite Luciferase Assay Substrate (lyophilized) 10 Vials
Stable-Lux Luciferase Assay System (100ml)	ASRV00014-100	Stable-Lux Luciferase Assay Buffer (100ml) Stable-Lite Luciferase Assay Substrate (lyophilized) 1 Vials

The Stable-Lux Luciferase Assay System components are stored based on their stability to maximize shelf life and usability. Transport conditions require temperatures >0℃.

Reagent Components (Unmixed):

- Long-Term Storage: Long-term storage for all components is recommended at -30℃ to -15℃
- Buffer Storage: The Stable-Lux Luciferase Assay Buffer can be stored for a long-term at room temperature (less than 25℃) or at 2℃ to 8℃.
- Substrate Stability: The Stable-Lux Luciferase Assay Substrate can be conserved for 30 days at 2℃ to 8℃.

Working Reagent (Once Mixed):

- Short-Term Stability: The mixed Steady-Lux Detection Reagent can be preserved for 1 day at room temperature (retaining greater than 80 percent activity) or at 2℃ to 8℃ (retaining greater than 90 percent activity).
- Mid-Term Storage: Unused working reagent can be preserved at -20℃ for 14 days (retaining greater than 90 percent activity).
- Long-Term Storage: It is recommended to preserve unused working reagent at -70℃ under conditions of long-term non-use.

Freeze-Thaw Tolerance:

- The system maintains stability after up to 10 cycles of repeated freezing and melting

Additional Equipment Required:

- Single/multi-channel pipettor
- Opaque-walled multiwell plates (e.g., white or black plates)
- Microplate reader with a luminescence detection module
- Orbital plate shaker
- 22℃ waterbath

3. Protocol

Reagent Preparation

1. **Thawing**: Incubate the Stable-Lux Luciferase Assay Buffer at 2 to 8℃ or room temperature to thaw. The product can also be incubated at 22℃ in a water bath but the temperature should not exceed 25℃.
2. **Preparation of Stable-Lux Luciferase Detection Reagent:** Add the entire bottle of thawed Stable-Lux Luciferase Buffer into the Stable-Lux Luciferase Substrate. Gently invert and mix it well for 3 to 5 times until the substrate is dissolved thoroughly.
3. **Equilibrate the Stable-Lux Luciferase Detection Reagent** to room temperature before use
4. **Mix**: Gently invert 5 times before use, to mix the solution evenly.

Detection steps

1. Remove the cell culture plate to be tested from the incubator and equilibrate at room temperature for 30 min.
2. Add equal amount of Stable-Lux Luciferase Detection Reagent which is equal to the volume of the cell culture to be tested. For example, when using a 96-well culture plate, add 100 µl Stable-Lux Luciferase Detection Reagent to 100 µl cell culture to be tested.
3. Incubate at room temperature for 5 minutes to lyse the cells.
4. Detect luminescence.

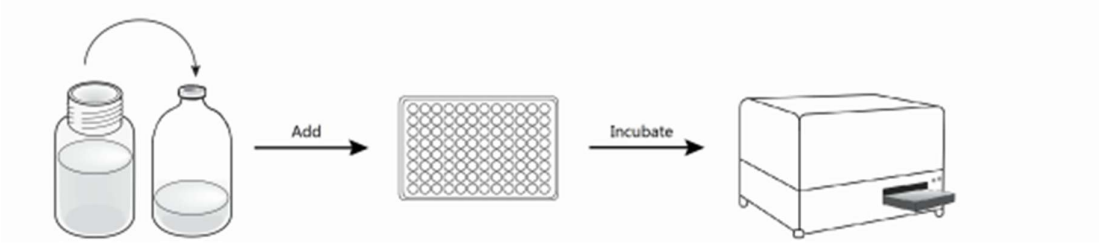

Figure 2. Short Protocol for Stable-Lux. The assay utilizes a two-step preparation followed by a homogeneous "Add-Mix-Read" detection. First, the Stable-Lux Luciferase Assay Buffer and Substrate are combined to create the working Stable-Lux Luciferase Detection Reagent. This ready-to-use reagent is then added in an equal volume directly to the cell culture. The reagent immediately induces cell lysis and releases the luciferase enzyme. Following a 5-minute incubation period at room temperature to ensure complete lysis and signal stabilization, the ultra-stable luminescence is measured.

Additional Protocol Considerations

1. **Temperature Equilibration:** Temperature control is critical, as both the luminescence intensity and the rate of decay are directly dependent on the reaction rate of the thermostable luciferase. To ensure the consistency and reliability of your assay results, it is mandatory that both the Stable-Lux detection reagent and the cell culture plate are fully equilibrated to room temperature (RT) before adding to the samples. Batch Operation Note: When processing multiple plates or using stacked plates, allow extra time for equilibration compared to single, monolayer plates. Inadequate temperature balance can lead to an uneven temperature distribution across the plate (a "gradient effect" between the center and edge), which introduces variability and inconsistency in test results.
2. **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 and allow for microscopic visualization of cell growth. 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. 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.

Notes:

Notes:

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.