TECHNICAL MANUAL

Mono-Lux Luciferase Assay System

- **SKU CODES:** ASRV00015-10 / ASRV00015-10x10 /ASRV00015-100
- **SIZE:** 10ml / 10x10ml / 100ml
- **DETECTION PRINCIPLE:** Luminescence
- **RUO:** Research-Use-Only

Revised: 11/2025

Mono-Lux Luciferase Assay System

Please read entire manual carefully before starting experiment.

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1. Product Description

The Mono-Lux Luciferase Assay System is a homogeneous, single-reagent solution designed for the ultrahigh sensitivity and stable quantitation of firefly luciferase reporter gene expression in mammalian cells. This robust system is ideally suited for high- and ultrahigh-throughput (UHTS) applications and batch processing of 96- and 384-well plates, delivering a stable optical signal upon direct addition to cultured cells.

The Mono-Lux system contains high-purity Luciferin and an optimized reaction reagent formulation. This proprietary chemistry provides enhanced stability, greater tolerance to environmental factors and sample components, and results in less peculiar smell compared to standard luciferase assay reagents.

Mechanism, Speed, and Stability

The **Mono-Lux Detection Reagent** facilitates a homogeneous, single-step reaction:

1. **Homogeneous Lysis:** Upon direct addition of an equal volume of mixed reagent to the cell culture medium, the mixed reagent induces cell lysis, releasing the luciferase enzyme.
2. **Fast Detection:** The luminescence signal is ready for measurement quickly; detection can be performed just 3 minutes after adding the mixed reagent.
3. **Signal Performance:** The kit generates high signal intensity with a reliable half-life period of 55 minutes, ensuring ample stability for batch processing. Importantly, the signal output is not affected by enzyme concentration, making it ideal for detection requiring maximum sensitivity.

Assay Advantages

- **Convenient Preparation:** The system is supplied as a two-component kit (solution and substrate) that is easily mixed to form the working reagent.
- **No Sample Preprocessing:** The homogeneous format means there is no need to remove culture medium or wash cells prior to adding the reagent, eliminating many handling inconveniences experienced in HTS.

- **Ultrahigh Sensitivity:** The optimized formulation provides ultrahigh sensitivity and a long-lasting, bright light output, especially beneficial for extended incubations.
- **Improved Precision:** The Mono-Lux Reagent is less sensitive to mixing and dispensing conditions, significantly enhancing reproducibility, making it ideally suited for high-density microplates (384- and 1536-well).
- **Reduced Interference:** The reagent is more stable and tolerant to sample components, including culture media, phenol red, and potential luciferase inhibitors than traditional reporter assays.

A Great High-Performance Alternative

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

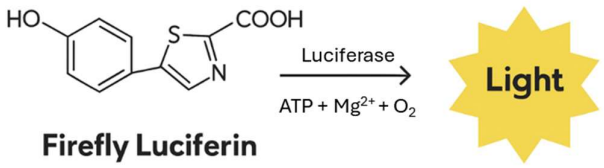

Figure 1. Schematic of Mono-Lux Detection Principle. The mono-oxygenation of luciferin is catalyzed by the thermostable luciferase in the presence of magnesium ions (Mg^{2+}), molecular oxygen (O_2), and ATP. The ATP required for this reaction is contributed directly by the viable cells following reagent-induced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

2. Kit Contents & Storage

Product	Code	Contents
Mono-Lux Luciferase Assay System (10ml)	ASRV00015-10	Mono-Lux Luciferase Assay Buffer (10ml) Mono-Lux Luciferase Assay Substrate (lyophilized) 1 Vial
Mono-Lux Luciferase Assay System (10x10ml)	ASRV00015-10x10	Mono-Lux Luciferase Assay Buffer (10x10ml) Mono-Lux Luciferase Assay Substrate (lyophilized) 10 Vials
Mono-Lux Luciferase Assay System (100ml)	ASRV00015-100	Mono-Lux Luciferase Assay Buffer (100ml) Mono-Lux Luciferase Assay Substrate (lyophilized) 1 Vial

The Mono-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 Mono-Lux Luciferase Assay Buffer can be stored at room temperature or at 2℃ to 8℃ for 90 days.
- **Substrate Stability:** The Mono-Lux Luciferase Assay Substrate can be stored for 21 days at Room Temperature or at 2℃ to 8℃ for 90 days (<85% activity).

Working Reagent (Once Mixed)

- **Short-Term Stability:** The mixed Mono-Lux Detection Reagent can be preserved for 1 day at room temperature (retaining greater than 80 percent activity) or at 2℃ to 8℃ for 5 days (retaining greater than 90 percent activity).
- **Mid-Term Storage:** Unused working reagent can be preserved at -20℃ for 60 days.

- **Long-Term Storage:** It is recommended to preserve unused working reagent at -70℃ under conditions of long-term non-use.

General Stability and Transport

- **Freeze-Thaw Tolerance:** The system maintains stability after up to 10 cycles of repeated freeze/thaw.
- **Transport Conditions:** The product can be transported at -20℃ to 0℃.

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 Mono-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 Mono-Lux Luciferase Detection Reagent:** Add the entire bottle of thawed Mono-Lux Luciferase Buffer into the Mono-Lux Luciferase Substrate. Gently invert and mix it well for 3 to 5 times until the substrate is dissolved thoroughly.
3. **Equilibrate the Mono-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 Mono-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 Mono-Lux Luciferase Detection Reagent to 100 µl cell culture to be tested.
3. Incubate at room temperature for at least 3 minutes to lyse the cells.
4. Detect luminescence.

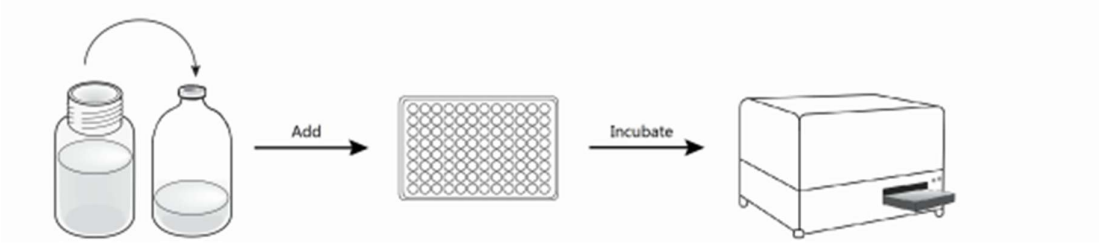

Figure 2. Short Protocol for Mono-Lux. The assay utilizes a two-step preparation followed by a homogeneous "Add-Mix-Read" detection. First, the Mono-Lux Luciferase Assay Buffer and Substrate are combined to create the working Mono-Lux Luciferase Detection Reagent. This ready-to-use reagent is then added in an equal volume directly to the cell culture. The reagent immediately induces cell lysis and releases the luciferase enzyme. Following at least a 3-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 Mono-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.

3. **Luminometers**: The Mono-Lux Luciferase Assay System is fully compatible with a microplate reader equipped with a luminescence detection module. Due to inherent differences in optical settings and sensitivity across various microplate reader models, the absolute measured luminescence signal values will naturally vary. Researchers should be aware that reader model differences may also influence the optimal detection window and settings required for the assay.

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:

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.