



## TECHNICAL MANUAL

### Bio-Lux Luciferase Assay System

- **SKU CODES:** ASRV00013-10 / ASRV00013-10x10 / ASRV00013-100
- **SIZE:** 10ml / 10x10ml / 100ml
- **DETECTION PRINCIPLE:** Luminescence
- **RUO:** Research-Use-Only

# Bio-Lux Luciferase Assay Systems

*Please read entire manual carefully before starting experiment.*

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## 1. Product description

The Bio-Lux Luciferase Assay System is a robust, highly sensitive, and homogeneous reagent solution specifically designed for the stable quantitation of firefly (*Photinus pyralis*) luciferase reporter gene expression in demanding applications like Reporter Bioassays. This system is optimized for batch processing of 96- and 384-well plates, delivering long-lived luminescence when added directly to cultured cells.

The Bio-Lux system contains high-purity Luciferin and an optimized reaction reagent formulation. This chemistry results in enhanced stability and greater tolerance to sample components and environmental factors than traditional luciferase assay reagents. The system is supplied as a two-component kit (solution and substrate) that is mixed prior to use.

### Mechanism, Speed, and Stability

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

1. **Homogeneous Lysis:** Upon direct addition of an equal volume of mixed reagent to the cell culture medium, the reagent immediately induces cell lysis, releasing the intracellular luciferase enzyme into the medium.
2. **Fast Detection:** The luminescence signal can be measured just 3 minutes after adding the mixed reagent.
3. **Signal Performance:** The intensity of the signal produced by the kit is high, and the half-life period is typically 55 minutes, which ensures sufficient stability for batch processing. Importantly, the signal is not affected by enzyme concentration.

### Potential Applications

The Bio-Lux assay is highly versatile and engineered for robust performance in demanding bioassays, including:

- **Fc Effect Detection** (e.g., ADCC Reporter Bioassays).
- **Immunology:** T-cell Activation Detection & Immune Checkpoint Detection.

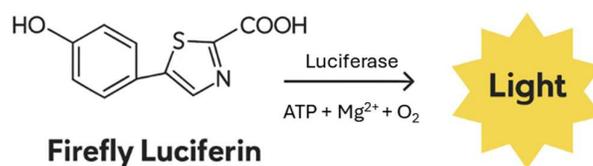
- **Drug Screening:** High-throughput compound screening where sample media interference is a concern.

### Assay Advantages

- **Enhanced Sample Tolerance:** The reagent is less sensitive to common sample components, culture media, and potential luciferase inhibitors than standard luciferase assays.
- **Simplified Assay Protocol:** The no-wash, no-pre-lysis format simplifies the workflow.
- **Improved Precision:** The reagent is less susceptible to variances in mixing and dispensing conditions, thereby enhancing reproducibility and reliability for bioassay applications.
- **Convenient Preparation:** The kit contains two components (solution and substrate) that are easily mixed to create the working reagent.

### A Great High-Performance Alternative

The Bio-Lux Luciferase Assay system is a great alternative to Promega Bio-Glo® and Promega Luciferase Assay System.



**Figure 1.** Schematic of Bio-Lux Detection Principle. The mono-oxygenation of luciferin is catalyzed by the thermostable luciferase in the presence of magnesium ions (Mg<sup>2+</sup>), molecular oxygen (O<sub>2</sub>), 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
Bio-Lux Luciferase Assay System (10ml)	ASRV00013-10	Bio-Lux Luciferase Assay Buffer (10ml) Bio-Lux Luciferase Assay Substrate (lyophilized) 1 Vial
Bio-Lux Luciferase Assay System (10x10ml)	ASRV00013-10x10	Bio-Lux Luciferase Assay Buffer (10x10ml) Stable-Lite Luciferase Assay Substrate (lyophilized) 10 Vials
Bio-Lux Luciferase Assay System (100ml)	ASRV00013-100	Bio-Lux Luciferase Assay Buffer (100ml) Stable-Lite Luciferase Assay Substrate (lyophilized) 1 Vial

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

### Reagent Components (Unmixed)

Reagent Components (Unmixed):

- **Long-Term Storage:** Long-term storage for all components is recommended at -30°C to -15°C
- **Buffer Storage:** Can be stored at **room temperature for 90 days** (retaining greater than 90 percent activity) or at 2°C to 8°C long-term.
- **Substrate Stability:** The Bio-Lux Luciferase Assay Substrate can be preserved at room temperature for 21 days or at 2°C to 8°C for 90 days (retaining greater than 90 percent activity)

### Working Reagent (Once Mixed)

- **Short-Term Stability:** The mixed Bio-Lux Detection Reagent can be preserved for 1 day at room temperature (retaining greater than 80 percent activity) or at or at 2°C to 8°C for 5 days (retaining greater than 85 percent activity).
- **Mid-Term Storage:** Unused working reagent can be preserved at -20°C for 60 days.

- **Long-Term Storage:** It is recommended to preserve unused working reagent at -70°C 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°C to 0°C.

#### **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°C waterbath

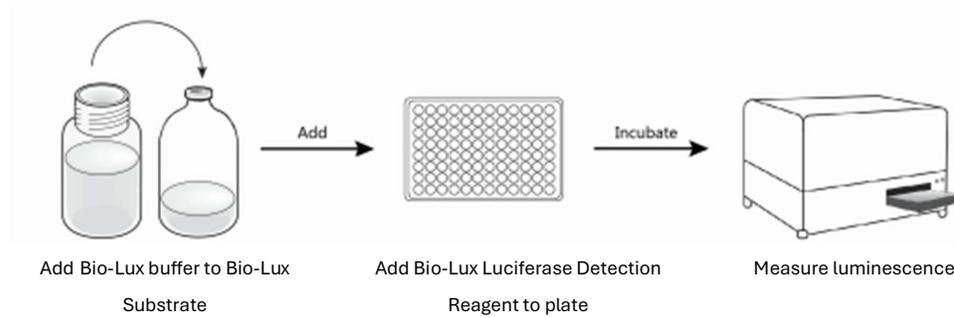
### 3. Protocol

#### Reagent Preparation

1. **Thawing:** Incubate the Bio-Lux Luciferase Assay 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 Bio-Lux Luciferase Detection Reagent:** Add the entire bottle of thawed Bio-Lux Luciferase Buffer into the Bio-Lux Luciferase Substrate. Gently invert and mix it well for 3 to 5 times until the substrate is dissolved thoroughly.
3. **Equilibrate the Bio-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 Bio-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 Bio-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 Bio-Lux.** The assay utilizes a two-step preparation followed by a homogeneous "Add-Mix-Read" detection. First, the Bio-Lux Luciferase Assay Buffer and Substrate are combined to create the working Bio-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 Bio-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 Bio-Lux Luciferase Assay System is fully compatible with any 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.

