TECHNICAL MANUAL

Nano-Lux Luciferase Assay System

- **SKU CODES:** ASRV00020-10 / ASRV00020-10x10 /ASRV00020-100
- **SIZE:** 10ml / 10x10ml / 100ml / 10 x 100 ml
- **DETECTION PRINCIPLE:** Luminescence
- **RUO:** Research-Use-Only

Revised: 07/2026

Nano-Lux Luciferase Assay System

Please read entire manual carefully before starting experiment.

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1. Product Description

The **Nano-Lux Luciferase Assay System** is a highly sensitive, stable and homogeneous reporter assay designed for the detection of **Nano-Lux luciferase activity** in cultured cells. Its optimized formulation enhances the luminescent signal while suppressing background luminescence, providing a broad detection window for reliable measurement of Nano-Lux reporter expression.

Technology and Applications

The assay uses a simple **prepare–add–incubate–read** workflow. First, the Nano-Lux Luciferase Assay Substrate is mixed with the Nano-Lux Luciferase Assay Buffer at a 1:50 volume ratio to prepare the Detection Reagent. The prepared reagent is then added directly to the cell culture at a volume equal to the sample volume. It lyses the cells, releases intracellular Nano-Lux luciferase and initiates the luminescent reaction. Following a 3–5-minute incubation at room temperature, luminescence is measured using a microplate reader equipped with a luminescence detection module.

The generated luminescent signal has a half-life of up to 2 hours under suitable assay conditions, providing flexibility when measuring multiple samples or plates. As the Detection Reagent is added directly to the cell culture, there is no requirement to remove the culture medium or wash the cells before detection.

The Nano-Lux Luciferase Assay System can be used in Nano-Lux reporter-based studies investigating:

- Gene regulation
- Signal transduction
- Protein stability
- Viral infection and replication
- Promoter and pathway activity

Assay Advantages

- **High Sensitivity:** Optimized chemistry strengthens Nano-Lux luminescence while reducing background signals.
- **Wide Detection Window:** Supports the measurement of both low and high reporter expression levels.
- **Stable Light Output:** Provides a luminescent signal with a half-life of up to two hours.
- **Simple Homogeneous Workflow:** Add the reagent directly to cultured cells, incubate for 3–5 minutes, and measure luminescence.
- **No Cell Washing Required:** Cells can be cultured and assayed in the same multiwell plate.
- **Suitable for High-Throughput Assays:** Compatible with opaque multiwell plates and automated liquid-handling workflows.

A Great High-Performance Alternative

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

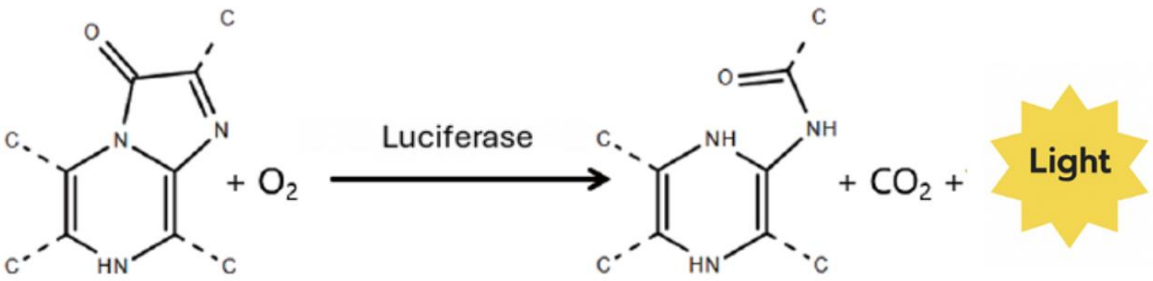

Figure 1. Nano-Lux Luciferase Assay Principle. NanoLux luciferase catalyses the oxidation of its substrate in the presence of molecular oxygen, generating a luminescent signal. The emitted light intensity is proportional to the amount of NanoLux luciferase present in the sample.

2. Kit Contents & Storage

Product	Code	Contents
Nano-Lux Luciferase Assay System (10ml)	ASRV00020-10	Nano-Lux Luciferase Assay Buffer (10ml) Nano-Lux Luciferase Assay Substrate (10ml)
Nano-Lux Luciferase Assay System (10x10ml)	ASRV00020-10x10	Nano-Lux Luciferase Assay Buffer (10x10ml) Nano-Lux Luciferase Assay Substrate (10x10ml)
Nano-Lux Luciferase Assay System (100ml)	ASRV00020-100	Nano-Lux Luciferase Assay Buffer (100ml) Nano-Lux Luciferase Assay Substrate (100ml)
Nano-Lux Luciferase Assay System (10x100ml)	ASRV00020-10x100	Nano-Lux Luciferase Assay Buffer (10x100ml) Nano-Lux Luciferase Assay Substrate (10x100ml)

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

Storage Conditions:

- Store all Nano-Lux Luciferase Assay System components at **−30°C to −15°C**. Transport the kit at **0°C or below**.
- The Nano-Lux Luciferase Assay Substrate remains liquid at the recommended storage temperature. Before opening the vial, briefly centrifuge it to collect any liquid that may have accumulated on the vial wall or cap.
- The substrate contains volatile components. Close the vial tightly immediately after use to minimise evaporation.

Working Reagents:

- Prepare the Nano-Lux Luciferase Detection Reagent immediately before use by combining the Substrate and Buffer at a **1:50 volume ratio**.
- Prepare only the amount required for the experiment. Storage of the prepared Detection Reagent is not recommended.

Additional Equipment Required:

- Single- or multichannel pipette
- Sterile pipette tips
- White or black opaque multiwell cell-culture plates
- Microplate reader equipped with a luminescence detection module
- Centrifuge suitable for brief centrifugation of reagent vials
- Room-temperature water bath, where required

3. Protocol

Reagent Preparation

1. **Thawing**: Remove the Nano-Lux Luciferase Assay Buffer from storage. Thaw the Buffer either:
    a. In a room-temperature water bath not exceeding 25°C; or
    b. Overnight at 2–8°C.

    Allow the Buffer to equilibrate fully to room temperature before preparing the Detection Reagent. The Nano-Lux Luciferase Assay Substrate remains liquid at the recommended storage temperature and does not require thawing.
2. **Preparation of Nano-Lux Luciferase Substrate:** Briefly centrifuge the Nano-Lux Luciferase Assay Substrate vial before opening to collect the complete contents at the bottom of the vial.
3. **Prepare the Nano-Lux Luciferase Detection Reagent:** Combine the Nano-Lux Luciferase Assay Substrate and Nano-Lux Luciferase Assay Buffer at a **1:50 volume ratio**. For example, to prepare approximately 10 mL of Detection Reagent, add:
    a. 200 µL Nano-Lux Luciferase Assay Substrate
    b. 10 mL Nano-Lux Luciferase Assay Buffer

4. **Mix**: Mix gently and thoroughly. Avoid vigorous shaking or introducing bubbles into the solution.

   Prepare the Detection Reagent fresh before use.

Detection steps

1. Remove the cell-culture plate from the incubator and allow it to equilibrate at room temperature for approximately **10 minutes**.
2. Add a volume of Nano-Lux Luciferase Detection Reagent equal to the volume of the test sample already present in each well. For example: add 100 µL Detection Reagent to 100 µL cell culture; or add 50 µL Detection Reagent to 50 µL cell culture. Ensure that the same reagent-to-sample ratio is used consistently throughout the plate.
3. Allow the plate to stand at room temperature for 3–5 minutes. During this incubation, the Detection Reagent lyses the cells, releases intracellular Nano-Lux luciferase and initiates the luminescent reaction.
4. Measure the luminescent signal using a microplate reader equipped with a luminescence detection module. For optimal sensitivity, use an open or full-spectrum luminescence detection setting where supported. When a defined wavelength is required, detection may be performed at approximately 460 nm.

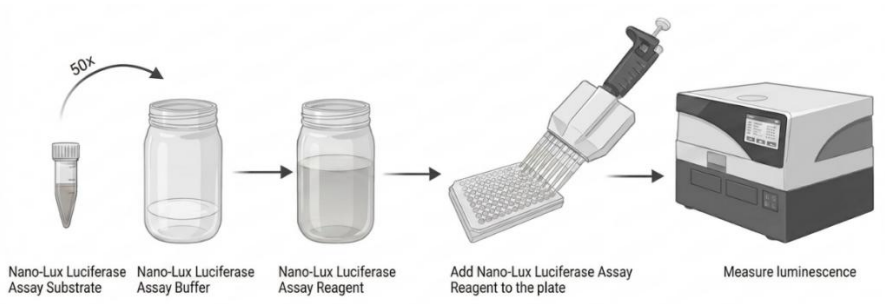

Figure 2. Short Protocol for Nano-Lux: The Nano-Lux Luciferase Assay System uses a simple homogeneous **prepare–add–incubate–read** workflow. First, combine the Nano-Lux Luciferase Assay Substrate and Buffer at a 1:50 volume ratio to prepare the Detection Reagent. Add the prepared reagent directly to the cell culture at a volume equal to the sample volume. Incubate the plate at room temperature for 3–5 minutes before measuring luminescence using a compatible microplate reader..

Additional Protocol Considerations

1. **Temperature Equilibration:** Temperature can influence the intensity and stability of the luminescent signal. Ensure that the Nano-Lux Luciferase Detection Reagent and the cell-culture plate are fully equilibrated to room temperature before combining them. When processing multiple or stacked plates, allow sufficient time for all plates to reach the same temperature. Uneven temperature distribution may introduce variability between wells or plates.

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. **Microplate Reader Settings:** Luminescence measurements may vary between microplate readers because of differences in instrument sensitivity, detector configuration, integration time and gain settings. Optimise the reader settings for the experimental system and keep them consistent across all samples and experimental groups. Avoid detector saturation when analysing samples with high Nano-Lux expression. Where necessary, reduce the number of cells, decrease reporter expression, shorten the integration time or dilute the sample.

4. **Signal Stability:** The luminescent signal has a half-life of up to 2 hours under suitable assay conditions. However, very high Nano-Lux expression may result in rapid consumption of the substrate and a shorter signal half-life. To improve signal stability, avoid excessive luciferase expression and ensure that samples are measured using consistent incubation and reading times.

5. **Experimental Control:** Include appropriate controls in every experiment, such as:
    - Cells without the Nano-Lux reporter construct
    - Untransfected or mock-transfected cells
    - Reagent-only background wells
    - Untreated control samples
    - Positive controls with confirmed Nano-Lux expression
    - Subtract the background signal from reagent-only or negative-control wells where appropriate.

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.