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Product Information

Luciferase Reporter Gene Detection Kit

Catalog Number **LUC1**
Storage Temperature –70 °C

TECHNICAL BULLETIN

Product Description

Firefly luciferase is one of the most commonly utilized reporter genes for the study of gene expression. It is an extremely sensitive, rapid, and easy to use procedure.[1,2] The chemiluminescent reaction catalyzed by luciferase is one of the most sensitive analytical tools for measuring gene expression identified.[3] Because of the nature of the luciferase protein, its activity is directly measurable in *in vitro* translation, and in eukaryotic and prokaryotic transfection systems.[4,5]

The Luciferase Assay Substrate includes coenzyme A, ATP, and luciferin. In the presence of ATP, luciferase catalyzes the oxidation of luciferin and generates a photon.[6] By adding coenzyme A to the reaction, light emission is more constant (half life >5 minutes) than conventional reactions in which a sharp spike of light is emitted followed by rapid decay.[7] This eliminates the need for automated luminometer injection of substrate and allows analysis by photographic film or scintillation counting.

The 5× Cell Culture Lysis Reagent contains Polymyxin B (PMB).[8] PMB is a strongly cationic antibiotic that disrupts the outer membrane of some Gram-negative bacteria[9] and increases the permeability of *E. coli*.[10] This eliminates the use of lysozyme, which must be made fresh before each use, and does not require freeze-thawing the cells to facilitate lysis. The 5× cell culture lysis reagent is also compatible with β-galactosidase assays.

Components

This kit is sufficient for 100 luciferase activity assays.

Luciferase Assay Substrate Lyophilized powder Catalog Number L0407	7 mg
Luciferase Assay Buffer Catalog Number L0532	10 ml
5× Cell Culture Lysis Reagent Catalog Number C4707	30 ml

Reagents Required but Not Provided

- Luciferase, Catalog Number L9506
- Bovine Serum Albumin (BSA), Catalog Number B4287
- Phosphate Buffered Saline (PBS), Catalog Number P4417

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store the kit at –70 °C

Reconstituted luciferase assay substrate is stable for 1 month at –20 °C or 1 year at –70 °C.

Procedures

I. Luciferase Standard Curve

Determine the linear range of the luminometer to be used as follows:

1. Produce a standard curve of light emission versus enzyme concentration (light units/ml), by serially diluting 1 volume of luciferase standard at ~4,000 light units/ml (Catalog Number L9506) to 1 volume of 1× Cell Culture Lysis Reagent containing 1 mg/ml BSA. In our laboratories, using a luminometer, the linear range was found to be between 2,000 and 10 light units/ml. The above may require adjustment to accommodate the sensitivity of the instrument being used.

2. Proceed to Luciferase Assay (Section III)

II. Luciferase Sample Preparation

A. Monolayer Cells (adapted from Current Protocols in Molecular Biology)[1]

1. Wash the cells directly on the culture dish by removing the medium and rinsing 3 times with an appropriate amount (4 ml for a 60 mm dish or ~0.14 ml/cm^2 of surface area) of ice cold 1× PBS. Aspirate after each wash.

2. Layer 1× Cell Culture Lysis Reagent over the cells (350 µl for a 60 mm dish or 12.5 µl/cm^2 of surface area) and incubate at room temperature for 15 minutes.

3. Scrape the cells into a microcentrifuge tube and centrifuge at 12,000 × *g* for 1 minute at 4 °C. Remove the supernatant and store on ice.

4. Proceed to Luciferase Assay (Section III).

B. Suspension Cells

1. Isolate the cells by centrifugation. Resuspend the pellet in 333 µl of 1× Cell Culture Lysis Reagent per ml of original culture. Incubate for 10 minutes.

2. Pellet cell debris by centrifuging at 12,000 × *g* for 1 minute at 4 °C. Remove the supernatant and store on ice.

3. Proceed to Luciferase Assay (Section III).

III Luciferase Assay

Note: Before use, resuspend the lyophilized Luciferase Assay Substrate in 10 ml of Luciferase Assay Buffer. Aliquot the solution in volumes that minimize freeze-thaw cycles and store at –70 °C.

1. Allow the luciferase substrate and cell lysate containing luciferase to equilibrate to room temperature before use.

2. Add 20 µl of the cell lysate or luciferase standard to 100 µl of the luciferase substrate. Mix well.

3. Begin reading light emission 10 seconds after mixing. The light intensity is nearly constant for ~20 seconds. A slow decay follows with a half-life of 5 minutes. The light emission can be read in a luminometer, scintillation counter,[11] or be detected with photographic film.[12]

4. Based on the standard curve, gene expression can be quantified in terms of luciferase units.

References

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