

Luciferase Reporter Assay Kit

(Catalog #K801-200; Store kit at -20°C)

I. Introduction:

Firefly luciferase has been used as a sensitive reporter for studying gene regulation and function. Luciferase provides a 1000-fold increase in sensitivity in comparison to the standard chloramphenicol acetyltransferase (CAT) assay. Firefly luciferase catalyzes the oxidative carboxylation of luciferin, a reaction with the highest efficiency of any known bioluminescence reaction. The light emission from the reaction can be recorded using a luminometer. BioVision's Luciferase Reporter Assay Kit provides a simple means for detecting luciferase activity in transfected eukaryotic cells. For measurement of expressed luciferase in vitro, luciferase is first extracted from transfected cells through cell lysis. Then CoA, ATP, Mg²⁺ and buffer are added to the lysate (Inclusion of CoA yields a nearly constant light emission rather than typical flash kinetics). The luminescent reaction is then triggered by an injection of luciferin, and the emitted light is recorded by a luminometer. By providing CoA, along with ATP, Mg²⁺ in an optimized buffer solution, BioVision's Luciferase Reporter Assay Kit ensures maximal sensitivity, consistent light output, as well as convenience and consistency when working with multiple samples.

II. Kit Contents:

Component	K801-200	Part Number
Substrate A	1 vial	K801-200-1
Substrate B	1 vial	K801-200-2
Cell Lysis Buffer	100 ml	K801-200-3

III. General Consideration and Reagent Preparation:

- Reconstitute Substrate A & B: Add 20 ml of Cell Lysis Buffer to each vial and mix well. Store both substrates at -70°C after each use. Store Lysis Buffer at 4°C.
- Ensure that all reagents have reached room temperature before performing assays.
- The following protocol is designed for using with adherent cultures growing in 35-mm tissue culture plates. If you are using plates of different size, adjust the volume proportionally. Read entire protocol before starting experiments.

IV. Assay Protocol:

A. Preparation of Cell Lysate

1. Remove media from cell culture plates and rinse once with PBS.
2. Add 1 ml of PBS and collect cells from plates by scraping and then transfer to a 1.5 ml microcentrifuge tube. Spin cells at 5000 rpm for 3 min and remove PBS.
3. Resuspend cells in 200 µl Cell Lysis Buffer and incubate on ice for 5 min. Centrifuge at 14000 rpm for 1 min. Transfer extract (supernatant) to a fresh tube and use immediately, or store at -70°C.

B. Luciferase Assay:

1. Place 20 - 100 µl cell extract into an assay cuvet or microplate well. Be sure to use the same volume for each sample.

Note: The amount of extract required may vary depending on the luciferase expression level and the instrumentation used; the amount used should be adjusted to keep the signal within the linear range of the assay.

2. Add 100 µl Substrate A.
3. Within 10 min, inject 100 µl Substrate B. Read the signal immediately using a luminometer. Note: The time between adding substrate B and reading signal should be as short as possible (1-2 sec) and consistent from sample to sample.

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