Product Information

StayBrite Highly Stable ATP Bioluminescence Assay Kit

I. Kit Contents:

Components	K2181-200 200 assays	Part Number
Substrate A	1 vial	K2181-C-1
Substrate B	1 vial	K2181-C-2
Cell Lysis Buffer	100 ml	K2181-C-3

II. Introduction:

Firefly luciferase catalyzes the highly efficient oxidative carboxylation of luciferin. The light emission of this biolumninescence reaction can be detected by a luminometer. Firefly luciferase is a sensitive reporter for gene regulation and function study. The Luciferase Reporter Assay Kit offers a fast and easy way of sensitive way of detecting lucifeasue 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, Mg^{2+} and buffer are added to the lysate. Then the injection of luciferin triggers the luminescent reaction which the emitted light can be detected by luminometer. This Luciferase Reporter Assay includes CoA, ATP and Mg^{2+} in an optimized buffer solution, to ensure highest sensitivity, consistent light output , plus convenience and consistency for multiple samples.

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℃ after each use. Store Lysis Buffer at 4℃.

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℃.

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.

For research use only! Not to be used in humans.

Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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