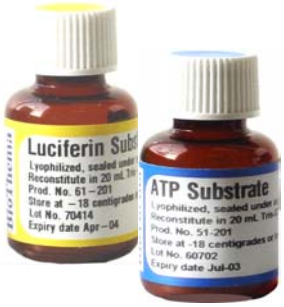
コスモ・バイオ株式会社

484-102 LUCIFERASE ASSAY HTS KIT



Intended use

The Luciferase Assay Kit is intended for the optimised assay of luciferase activity *in vitro* in reporter gene studies. The kit is customised for HTS use in a microplate format in a luminometer without dispensers.

Assay principles

Firefly luciferase catalyses the following reaction:

$$\text{ATP + D-luciferin + } O_2 \xrightarrow{\text{Luciferase}} \text{AMP + PPi + oxyluciferin + } CO_2 + \text{light}$$

The assay has been optimised to give maximum level of stable light (decay rate below 6%/min)[1]. In addition to the two substrates (ATP and D-luciferin) the reagents also contain magnesium ions, PPi (inorganic pyrophosphate), DTT (dithiothreitol) and BSA (bovine serum albumin).

Kit contents

The kit contains 24 vials of ATP Substrate, 24 vials of Luciferin Substrate and 1 L Tris-EDTA Buffer (0.1 mol/L tris(hydroxymethyl)aminomethane plus 2 mmol/L EDTA adjusted to pH7.75 with acetic acid). Each set of the lyophilised substrates allows 200 assays in 96-well microplate format (i.e. a total of 4800 assays).

Applications

Firefly luciferase is an ideal reporter for monitoring promoter activity in the control of gene expression for several reasons:

1. Firefly luciferase is not present in normal cells.
2. The assay is very sensitive and easy to perform.
3. Simple manual luminometers or fully automatic microplate luminometers can be used.
4. Suitable for HTS.

The kit is therefore a highly interesting alternative to CAT assays and other non-luminescent reporter gene assays.

Instrumentation

Any luminometer can be used. The detection limit obviously depends on the luminometer. With most luminometers 10^{-19} moles of luciferase can be detected.

Assay procedure

1. The cells must be lysed before the assay.
2. Add 10 µL of lysed sample to a cuvette or a microplate well.
3. Add 100-500 µL of Luciferin Substrate (reconstituted in 20 mL Tris-EDTA Buffer).
4. Add the same volume of ATP Substrate (reconstituted in 20 mL Tris-EDTA Buffer).
5. Measure the light emission.

[1]A. Lundin (1993) Optimised assay of firefly luciferase with stable light emission. In Bioluminescence and Chemiluminescence (A. Szalay, L. Kricka and P. Stanley, Eds.), pp. 291-295, John Wiley & Sons, Chichester)