

Luciferase Assay Kit

Optimised *in vitro* assay of luciferase activity in reporter gene studies



- Fast assay: Results within minutes
- User-friendly: HTS kits available
- Highly sensitive: 10^{-19} mol luciferase
- Flexible: Choice of μ -plate & cuvette methods

Leader in luminescent
ATP-assays

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LUMINESCENT ASSAYS 

Luciferase Assay Kit

Intended use

The Luciferase Assay Kit is intended for the optimised assay of luciferase activity *in vitro* in reporter gene studies. This assay is also available in two versions for high throughput screening (HTS). Please inquire.

Applications

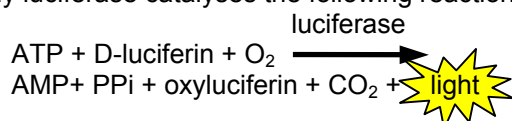
Firefly luciferase is an ideal reporter for monitoring promoter activity in the control of gene expression for several reasons:

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.

Assay principles

Firefly luciferase catalyses the following reaction:



The assay has been optimised¹ to give maximum level of stable light (decay rate 2-4%/min depending on type of luciferase). 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).

Instruments

The stable light makes it possible to use manual single tube luminometers as well as microplate luminometers. The detection limit obviously depends on the luminometer. With most luminometers 10⁻¹⁹ moles of luciferase can be detected.

Kit contents

The kit consists contains 8 sets of reagents and each set allows 100 assays in a 96-well microplate (i. e. a total of 800 assays).

1. ATP Substrate. 8 vials of lyophilised reagent containing ATP and stabilisers.
2. Luciferin Substrate. 8 vials of lyophilised reagent containing D-luciferin and stabilisers.
3. Tris-EDTA Buffer. 2x100 mL 0.1 mol/L Tris(hydroxymethyl) aminomethane containing 2 mmol/L EDTA and adjusted to pH 7.75 with acetic acid.

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 reconstituted Luciferin Substrate.
4. Add the same volume of reconstituted ATP Substrate.
5. Measure the light emission.

¹A. 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).

Product characteristics

Detection limit: 10⁻¹⁹ mol luciferase
No. of determinations (cuvettes): 160
No. of determinations (microplate): 800

Ordering info

Article No: 484-001