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

BioThema
LUMINESCENT ASSAYS

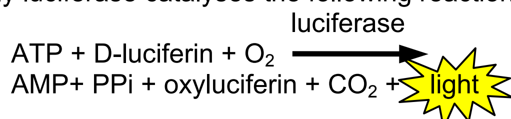
Luciferase Assay Kit

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 optimised[1] 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.

[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).

Product characteristics
Detection limit: 10^{-19} mol luciferase
No. of determinations (cuvettes): 160
No. of determinations (microplate): 800

Ordering info
Article No: 484-001

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Luciferase Assay Kit Prod. No. 484-001

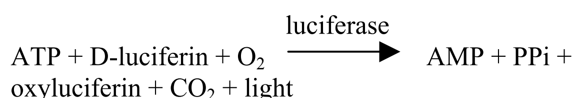
Instructions for use

Intended use

The Luciferase Assay Kit is intended for the optimised assay of luciferase activity *in vitro* in reporter gene studies.

Assay principles

Firefly luciferase catalyses the following reaction:



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

The assay has been optimised to give maximum level of stable light (decay rate below 6%/min). 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) (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).

Kit contents

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The ATP and Luciferin Substrates should be stored at -18 ^{o}C. The Tris-EDTA Buffer may be stored at +4 ^{o}C. The kit and the individual components are labelled with expiry date.

Reagent reconstitution

The ATP Substrate is reconstituted by adding 12 mL Tris-EDTA Buffer to the vial. The Luciferin Substrate is reconstituted by adding 10 mL Tris-EDTA to the vial. Remove the rubber stopper with a clean pair of tweezers to avoid contamination. Use a clean disposable pipette tip. Once reconstituted the Luciferin Substrate must be protected from light.

Reconstituted reagents are stable for 8 hours at 25 ^{o}C and 1 month at -18 ^{o}C.

Instrumentation

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.

Assay procedure

1. The cells must be lysed before the assay. This can be achieved by a variety of methods. Please contact us if advice is needed.
2. The reconstituted reagents must be allowed to attain room temperature before the assay, since the optimum temperature of the luciferase reaction is approx. 25 ^{o}C.
3. Add 10 µL of lysed sample to a cuvette or a microplate well.
4. Add 100-500 µL of reconstituted Luciferin Substrate.
5. Add the same volume of reconstituted ATP Substrate.
6. Measure the light emission as soon as possible (maximum light emission is normally obtained within a few seconds and will then start to decay slowly).

Procedural notes

1. A high cell density in the sample may result in degradation of ATP and PPi causing a rapid decay of the light. This is easily detected by making two light readings. The first reading is done immediately after adding the Substrates and the second reading 1 min later. If you have this problem the sample should either be diluted or higher volumes of the Substrates should be used. The latter alternative is only possible, if a cuvette luminometer is used.

2. Some lysis buffers or cell media may have an inhibitory effect on the reaction. It is suggested to use a luciferase standard to compensate for such effects. If added as an internal standard this will also allow you to express your results in attomoles or femtograms of luciferase rather than relative light units (RLU). The latter unit does not allow comparisons between different labs using different luminometers or different assay kits.

3. If the luciferase in your sample has been partially inactivated (luciferase contains e. g. SH-groups that may be oxidised), it may take some time before the stabilisers in the reagents have fully activated all luciferase molecules. Thus it is good practice to check the time-course of the light emission, when working with stored samples or with new lysing methods. If you have a problem, you may try pre-incubation with Luciferin Substrate (do not pre-incubate with ATP Substrate, since ATP or PPi may be degraded). If this does not help, please, contact BioThema.

4. If maximum light is obtained e. g. 15 s after adding the Substrates, maximum accuracy is obtained by making the light reading immediately after 15 s. The recommended integration time for the light reading is 1-10 s. Increasing the integration time above 10 s makes very little difference in terms of detection limit.

5. Don't use ATP Substrate from one kit lot with Luciferin Substrate from another kit lot. The two substrates are quality controlled against each other and BioThema can't guarantee that the same luciferase activity is obtained with substrates that have not been tested against each other.