Firefly Luciferase Assay Kit

(1000 assays)

Zellbio GmbH (Germany)

CAT No. ZX-66118-1000

www.zellx.de

Application:

Quantitation of firefly luciferase reporter enzyme activity

!!! Caution: This product is for Research Use Only. Not for *in-vitro* Diagnostics !!!

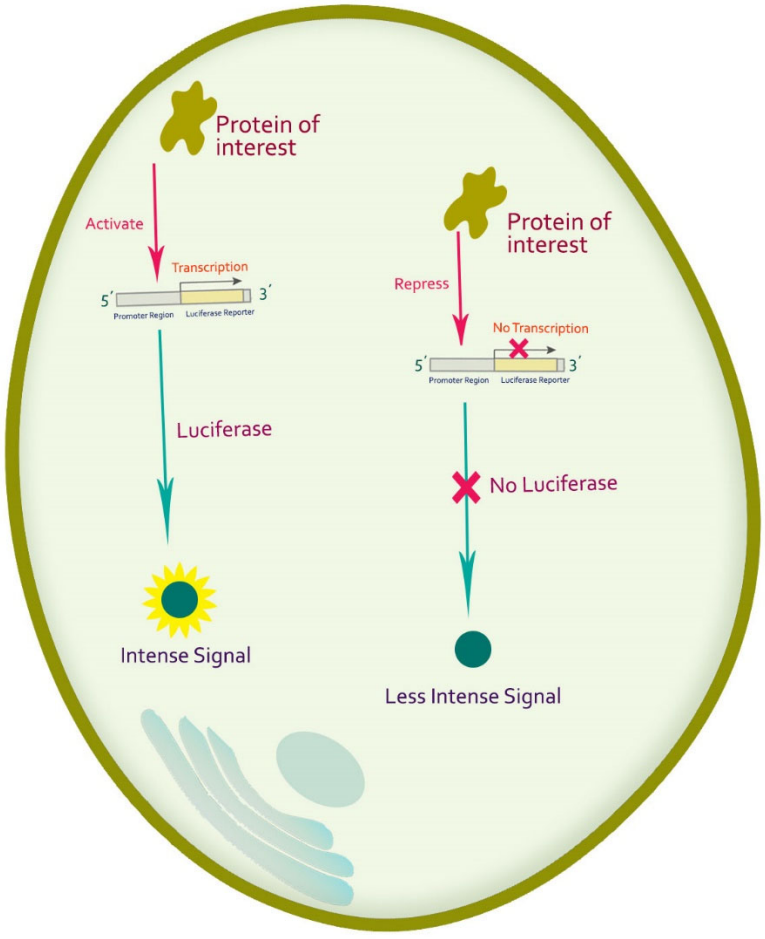
Table of Contents

Please read this insert completely prior to using the product.

Background

Firefly luciferase is a most abundantly used photoprotein in molecular biology and gene regulation studies such as gene expression, receptor activity, signal transduction, mRNA processing, protein folding and protein:protein interactions. Firefly luciferase or the luciferase from *Photinus pyralis*, is a monomeric protein with the molecular weight of 62 kDa which is active as a monomer and does not require subsequent processing for its activity. The Luciferase enzyme catalyzes the oxidation of D-luciferin in an ATP-dependent manner in the presence of Mg2+ and oxygen to oxyluciferin, and is producing light emission centered at 560 nm that is directly proportional to the quantity of luciferase enzyme in the reaction mixture. Presence of coenzyme A in the reaction mixture enhances the sensitivity of the assay and provides a sustained light reaction (half-life >5 minutes).

ZellX® firefly luciferase assay kit is a luminescence assay designed for simple and efficient quantitation of firefly luciferase reporter enzyme activity from cultured cells with high sensitivity and linearity.



Intended use

Quantitation of firefly luciferase reporter enzyme activity from cultured cells.

Materials supplied in the Kit

Component	*Quantity*
Luciferase Assay Buffer (including D-Luciferin)	100 mL
Cell Lysis Concentrate (5X)	20 mL

Storage instruction

All reagents, should be stored at -80° C and protected from light until the expiration date of the kit. **Avoid freezing/thawing cycles**. For short term storage it can be kept at -20° C and protected from light (up to three months).

Materials required but not supplied

Precision pipettes and disposable filter pipette tips

Sterile white solid 96 well plate

Precautions

This kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

General remarks

- The instruction must be strictly followed.
- Pipette tips should not be used more than once to avoid cross contamination.
- Reagents of different batches should not be mixed or used after their expiration dates.

Reagent preparation

i. **Cell Lysis Solution (1X):** Prepare a 1:5 dilution of Cell Lysis Concentrate with diH2O (1 part Cell Lysis Conc. with 4 parts diH2O), and mix well. Cell Lysis Solution (1X) must be used immediately after preparation.

Assay Procedure

The procedure has been designed and validated for 96-well format. Other format requires scaling and optimization by the end-user.

- Each plate must be included at least two wells as background control which contain no cells and only 50 µL 1x Lysis Buffer.

1. Aspirate the culture medium from the wells gently and wash the cells with appropriate amount of PBS.
2. Remove the PBS and add 50 µL of 1x Lysis Buffer to each well.
3. Incubate with shaking for 15 minutes at room temperature (shaking can increase the lysing of the cells).
4. Transfer the lysate to the new tubes or plate (we recommend to use 96-Well Solid Plate (white))
5. Keep the plate or tubes at 4°C for until performing the assay. lysates must be stored at -20° C or -80° C if assay will not be performed on the same day.
6. Set up the luminometer for the appropriate delay and measurement times.
7. Add 100 µL of luciferase substrate solution to each well and shake for 30 seconds.
8. Immediately place the plate under the laminator and start reading luminescence signal.

Calculation

I. Average the duplicate luminescence signal for each sample.

II. Subtract the background luminescence signal from the samples signal to obtain normalized signal values.