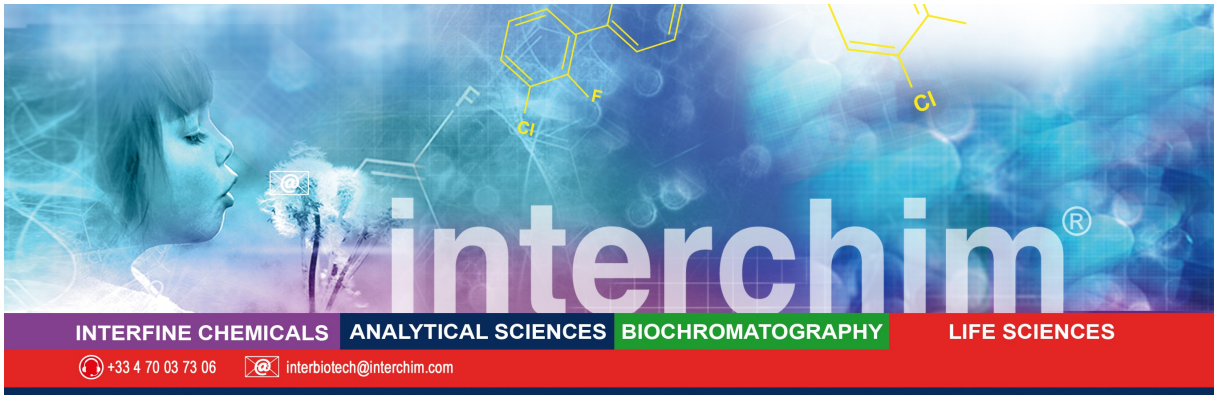
FT-BE7930



Renilla Luciferase Assay Kit

Product Description

Name : ***Renilla* Luciferase Assay Kit**

Catalog Numbers : FP-BE7930 150 tests
FP-BE7931 1000 tests

Product Components :

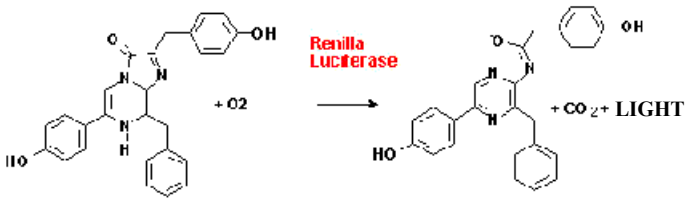
	150 tests	1000 tests
Coelenterazine native (lyophilized)	3 vials (50µg)	4vials (250 µg)
5X *Renilla* Luciferase Assay Lysis Buffer	10 ml	30 ml
Renilla Luciferase Assay Buffer	10 ml	50 ml
Renilla Luciferase Assay Enhancer	10 ml	50 ml

Storage: –70°C (Q) or –20°C (short term) Protect from light and moisture
The components of the kit are stable at -70°C for >6 months (3 months at -20°C). *Renilla* luciferase assay solution (Assay Buffer + Coelenterazine) should be prepared fresh for each use and used within 2 h for best results. Avoid repeated freeze-thaw cycles. Aliquot *Renilla* Luciferase Assay buffer an Enhancer for storage if necessary.

Introduction

Renilla Luciferase has been used as a reporter gene for studying gene regulation and function *in vitro* and *in vivo*. Recently, *Renilla* luciferase has been widely used in multiplex transcriptional reporter assays or as a normalizing transfection control for *Firefly* luciferase assay. *Renilla* luciferase, a monomeric 36 000 Dalton protein, catalyzes coelenterazine oxidation by oxygen to produce light. The enzyme does not require post-translational modification for its activity and may function as a genetic reporter immediately following translation. Coelenterazine native is the natural substrate for *Renilla* luciferase. However, over a dozen of coelenterazine analogs have been synthesized, many of which are now commercially available from FluoProbes. These coelenterazine analogs all function as substrates for *Renilla* luciferase with different properties in term of emission wavelength, cell membrane permeability, and quantum efficiency. Coelenterazine also emits light from enzyme-independent oxidation, a process known as autoluminescence. The autoluminescence is enhanced by superoxide anion and peroxynitrite in cells and tissues. *Renilla* Luciferase Assay Kit is designed to provide a simple and sensitive method of detecting *Renilla* luciferase. Through utilizing a special coelenterazine derivative and buffer

formulation, this assay kit is designed to yield reliable, linear results with minimal autoluminescence background and superior sensitivity. This kit is a flash-type luminescence assay, with signal half-life of about 2 minutes.



Bioluminescent reaction catalyzed by *Renilla* luciferase

Directions for use

Protocol - Preparation of Cell Lysates

A. Preparation of 1X Passive Lysis Buffer

1X Passive Lysis Buffer is prepared by adding 1 volume of 5X Passive Lysis Buffer to 4 volumes of distilled water and mixing well. The 1X Passive Lysis Buffer may be stored at 4°C for up to one month. Store the 5X Passive Lysis Buffer at -20°C.

B. Lysis of Cells Cultured in Multiwell Plates

1- Remove the growth medium from the cultured cells and gently add a sufficient volume of phosphate buffered saline (PBS) to wash the surface of the culture vessel. Add 1X Passive Lysis Buffer using the volume recommended below for each type of well:

6 well culture plate 500 µl
12 well culture plate 250 µl
24 well culture plate 100 µl
48 well culture plate 65 µl
96 well culture plate 20 µl

2- Place the culture plates on a rocking platform or orbital shaker with gentle rocking/shaking to ensure complete and even coverage of the cell monolayer with 1X Passive Lysis Buffer. Rock the culture plates at room temperature for 15 minutes.

Note: Cultures that are overgrown are often more resistant to complete lysis and typically require an increased volume of Passive Lysis Buffer and/or an extended treatment period to ensure complete lysis and/or scraping cells off the culture plates.

3- Transfer the lysate to a tube or vial and place in 4°C for further assay. Although it is not necessary, the lysate can be cleared by centrifugation for 30 seconds at top speed in a refrigerated microcentrifuge and transferred into a new tube. Store lysates at -70°C if not for immediate use.

Protocol - *Renilla* Luciferase Assay

A. Preparation of *Renilla* Luciferase Assay Solution

1. Prepare an adequate volume to perform the desired number of *Renilla* Luciferase Assays (50µL reagent per assay). Thaw a bottle of Renilla luciferase assay buffer and pipette the desired volume into clean container.
2. Prepare 1 mg/ml coelenterazine
   Resuspend 1 vial of Coelenterazine with 50 µL MeOH (for **150 tests kit**) or 1 vial of Coelenterazine with 250 µL MeOH (for **1000 tests kit**) to derive 100X stock.
3. Add 1volume of 1 mg/ml Coelenterazine stock to 50 volumes of *Renilla* Luciferase Assay Buffer to derive Renilla luciferase working solution. *Renilla luciferase* working solution (coelenterazine + Renilla luciferase assay buffer) should be prepared fresh and used within two hours. Store unused 1 mg/mL coelenterazine stock at -20°C.

FT-BE7930

B. Standard Protocol

For manual luminometer:

1- Set up luminometer with appropriate parameters (delay time, integration time and sensitivity, etc.).

2- Add 20µl of cell lysate into a luminometer tube.

3- Add 50µl of *Renilla* Luciferase Assay Enhancer into the tube, flick the tube a few times for thorough mixing.

4- Add 50µl of *Renilla* Luciferase working solution (assay buffer + coelenterazine) to the tube and mix quickly by vortexing or flicking the tube with a finger.

5- Place tube in luminometer and initiate measurement. Luminescence is normally integrated over 10 seconds without delay. Other integration times may also be used.

6- If the luminometer is not connected to a printer or computer, record the *Renilla* luciferase activity measurement.

7- Discard the reaction tube, and proceed to the next *Renilla* Luciferase Assay.

For luminometer with injector:

1- Format the luminometer so that the injector dispenses 50 µl. Prime the injector with *Renilla* Luciferase working solution (assay buffer + coelenterazine).

2- For each reaction, carefully add 20µl of cell lysate to an individual luminometer tube or to the wells of a multiwell plate.

3- Add 50 µL *Renilla* Luciferase Assay Enhancer into each reaction.

4- Place the samples in a luminometer.

5- Initiate measurement. This action will cause *Renilla* Luciferase Working Solution to be injected into the reaction vessel and the measurement to be subsequently taken. Luminescence is normally integrated over 10 seconds without pre-read delay. Other integration times may also be used.

6- Record the *Renilla* luciferase activity measurement.

7- If using a single tube luminometer, discard the reaction tube, and proceed to the next *Renilla* Luciferase Assay reaction. If using a plate luminometer, the luminometer will automatically begin injecting *Renilla* Luciferase Assay Solution into the next well indicated on the luminometer plate.

Determination of Background Luminescence

The expression of a luciferase reporter is quantified by the luminescence produced above background levels. In most cases, because the background created by the reagent in the absence of *Renilla* luciferase is very small compared to the luciferase signal, this luciferase activity is directly proportional to total luminescence. However, when measuring very small amounts of luciferase it is important to subtract the background signal from the measurement of total luminescence. Background luminescence can be obtained by using lysate from untransfected cells or cells transfected with a control vector. The background luminescence can be subtracted from subsequent measurements of *Renilla* luciferase.

References

- **Bhaumik S**., *et al.*, "Optical imaging of *Renilla* luciferase, synthetic *Renilla* luciferase, and firefly luciferase reporter gene expression in living mice.", *J Biomed Opt*., **9**, 578 (2004)
- **Matijasevic Z**., *et al.*, "Repair of sulfur mustard-induced DMA damage in mammalian cells measured by a host cell reactivation assay.", *Carcinogenesis*., **22,** 661 (2001)
- **Nieuwenhuijsen BW**., *et al.*, "A dual luciferase multiplexed high-throughput screening platform for protein-protein interactions.", *J Biomol Screen*., **8**, 676 (2004).
- **Matthews, J.C**., *et al.*, "Purification and properties *of Renilla* reniformisluciferase.", *Biochemistry*, **16**, 85 (1977)

Related products

- D-PBS, 1X USP Sterile, GS3576
- Luciferase Assay Kit, *Bright glow*, FP-JQ6811
- *Firefly* & *Renilla* Luciferase Assay Kit, FP-BE7810
- *Firefly HTS* Luciferase Assay Kit, FP-BU6870
- Growth plate 96x1ml, sterile BS6200
- Growth plate 96x2ml, sterile BS6210

Ordering information

Catalog size quantities and prices may be found at http://www.fluoprobes.com
Please inquire for higher quantities (availability, shipment conditions).

For any information, please ask : FluoProbes® / Interchim; Hotline : +33(0)4 70 03 73 06

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