Luciferase Reporter Assay Kit II (Renilla)

PromoKine

Instruction Manual

Product Name	Product Description	Size	Catalog Number
Luciferase Reporter Assay Kit II (Renilla)	Luciferase Reporter Assay Kit II (Renilla)	150 assays 1000 assays	PK-CA707-30004-1 PK-CA707-30004-2

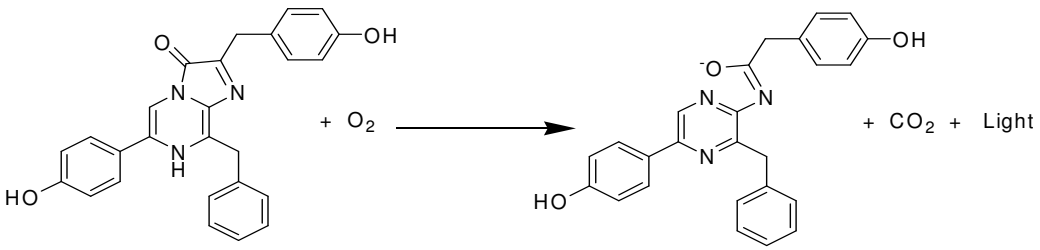
Introduction

Renilla Luciferase has been used as a reporter gene for studying gene regulation and function in vitro and in vivo[1, 2]. Recently, *Renilla* luciferase has been widely used in multiplex transcriptional reporter assays or as a normalizing transfection control for *Firefly* luciferase assay[2, 3]. *Renilla* luciferase, a monomeric 36,000 Dalton protein, catalyzes coelenterazine oxidation by oxygen to produce light[4] (Figure 1). 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 PromoKine. 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. PromoKine's Luciferase Reporter Assay Kit II (*Renilla*) 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 (Figure 2). This is a flash-type luminescence assay that requires signal to be measured immediately after adding working solution to samples. The luminescence signal decays over the course of about 2 minutes of reaction time, although signal half-life may vary depending on luciferase expression levels.

The Renilla Luciferase Assay features Aquaphile™ Coelenterazine, a water soluble substrate that can be stored at -20°C with minimal evaporation, unlike methanol solutions of coelenterazine.

PromoKine also offers the Luciferase Reporter Assay Kit III (Firefly & Renilla Single Tube Assay), a combined luciferase assay allowing sequential measurement of Firefly and Renilla luciferase activity in the same sample with high sensitivity and linearity (see related products).

Figure 1. Bioluminescent reaction catalyzed by *Renilla* luciferase.



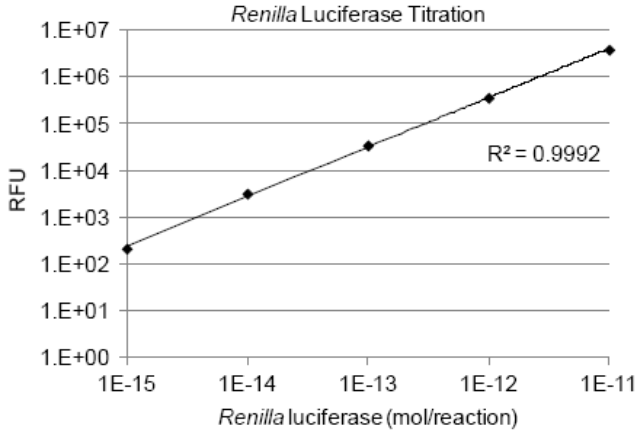


Figure 2. Titration of recombinant Renilla luciferase in the PromoKine *Renilla* Luciferase Assay. *Renilla* luciferase was serially diluted in 1X Passive Lysis Buffer and measured in the assay. Luminescence was measured on a Promega Glomax® 20/20 single tube luminometer with integration time of 1 second. Background from reagents without enzyme added was subtracted from luminescence values.

Kit Contents

Components:	PK-CA707-30004-1	PK-CA707-30004-2
	150 assays	*1,000 assays*
Aquaphile™ Coelenterazine, lyophilized	3 vials (3 x 200 µg)	1 vial (4 mg)
Passive Lysis Buffer, 5X	10 ml	30 ml
Renilla Luciferase Assay Buffer	15 ml	100 ml

Note: Enough lysis buffer is provided to perform the stated number of assays with cells grown in culture plate sizes ranging from 96-well to 24-well. For applications requiring more lysis buffer (see Assay Protocol), additional 5X passive lysis buffer (cat. no. PK-CA707-99912) may be purchased separately.

Storage and Stability

Store the kit at -80°C. Renilla Luciferase Assay Buffer is stable at -80°C for at least three months from date of receipt. Other kit components are stable at -20°C for at least six months from date of receipt. Kit components and stock solutions of Aquaphile coelenterazine in water are stable to at least 5 freeze/thaw cycles.

Assay Protocol

Preparation of Cell Lysates

A. Preparation of 1X Passive Lysis Buffer

1. Prepare 1X Passive Lysis Buffer by adding 1 volume of 5X buffer to 4 volumes of dH_2O and mixing well. 1X Passive Lysis Buffer may be stored at 4°C for up to one month.

B. Lysis of Cells Cultured in Multiwell Plates

2. Remove the growth medium from the cultured cells and gently wash the cells once with a sufficient volume of phosphate buffered saline (PBS) to cover the surface of the culture vessel. Remove the PBS and 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 250µL
    - 24 well culture plate 100 µL
    - 48 well culture plate 65 µL
    - 96 well culture plate 20 µL

3. 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. PromoKine offers mini cell scrapers (Cat.No. PK-CA707-22003) for harvesting lysates from 96-, 48-, and 24-well plates.

4. Transfer the lysate to a tube or vial. Place at 4°C until ready to assay. Store lysates at -20°C or -80°C if assay will not be performed on the same day.

Renilla Luciferase Assay

A. Preparation of *Renilla* Luciferase Working Solution

Thaw Renilla Luciferase Assay Buffer at room temperature.

Prepare 2 mg/mL Aquaphile™ coelenterazine stock solution. For component 10126-200ug, add 100 uL water to the vial and mix. For component 10126-4mg, add 2 mL water to the vial and mix. Stock solutions of Aquaphile coelenterazine can be stored for up to 3 months at -20oC or below.

Kit **PK-CA707-30004-1:** Add 100 µL water to **1 vial** (200 µg) and mix (= 2 mg/ml).

Kit **PK-CA707-30004-2:** Add 2 ml water to **1 vial** (4 mg) and mix (= 2 mg/ml).

Prepare enough *Renilla Working Solution* to perform the desired number of assays (100 µL working solution per assay). Dilute Aquaphile coelenterazine (2 mg/mL) in Renilla Luciferase Assay Buffer at a ratio of 1:50. For example, add 20 µL Aquaphile™ coelenterazine stock solution to 1 mL assay buffer. For best results, working solution (assay buffer with substrate) should be prepared fresh before each use, and used within 3 hours of preparation. *Renilla Working Solution* activity is stable for up to 3 hours, but background increases up to 60% after 5 hours at room temperature.

B. Standard Protocol

The protocol below is for manual assay using a single-tube luminometer. If your luminometer is equipped with an automatic injector, it may be used to dispense working solution into each luminometer tube or well of a multiwell plate according to the instructions for your instrument.

For manual luminometer:

1. Set up luminometer with parameters (delay time, integration time and sensitivity, etc.) recommended for your instrument for dual luciferase assay. We routinely use integration time of 1 second.
2. Add 20 µL of cell lysate into a reaction tube that is compatible with your luminometer.
3. Add 100 µL of *Renilla Working Solution* to the same reaction tube and mix by pipetting up and down several times or briefly vortexing.
4. Immediately place tube in luminometer and record the Renilla luminescence measurement.
5. Discard the reaction tube, and proceed to the next *Renilla* Luciferase reaction.

For luminometer with injector (protocol example):

1. Format the luminometer so that the injector dispenses 100 µL. Prime the injector with *Renilla Working Solution* (coelenterazine + assay buffer).
2. For each reaction, carefully add 20 µL of cell lysate to an individual luminometer tube or to the wells of a multiwell plate.
3. Add 100 µL of *Renilla Working Solution* into each reaction.
4. Place the samples in a luminometer.
5. Initiate measurement. This action will cause *Renilla 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 plate luminometer, the luminometer will automatically begin injecting *Renilla Working Solution* into the next well indicated on the luminometer plate.

Determination of Assay Backgrounds

The expression of a luciferase reporter is quantified by the luminescence produced above background levels. In most cases, background created by the reagent in the absence of luciferase is very low compared to signal with luciferase. However, when

measuring low levels of luciferase activity, it is important to subtract the background signal from untransfected cells or cells transfected with a negative control vector from measurements of luciferase activity.

Intended Use

For in vitro research use only. Not for diagnostic or therapeutic procedures.

References

1. Bhaumik S. et al. (2004) Optical imaging of Renilla luciferase, synthetic Renilla luciferase, and firefly luciferase reporter gene expression in living mice.J Biomed Opt. 9, 578-86.
2. Matijasevic Z. et al. (2001) Repair of sulfur mustard-induced DNA damage in mammalian cells measured by a host cell reactivation assay. Carcinogenesis. 22, 661-4.
3. Nieuwenhuijsen BW. et al. (2004) A dual luciferase multiplexed high-throughput screening platform for protein-protein interactions. J Biomol Screen. 8, 676-84.
4. Matthews, J.C., Hori, K. and Cormier, M.J. (1977) Purification and properties of Renilla reniformis luciferase. Biochemistry 16, 85–91.

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