Renilla Luciferase Assay Kit

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Catalog # PR300002 & PR300007

1. Introduction

Renilla luciferase has been used as a reporter gene for studying gene regulation and function in vitro and in vivo. It commonly is used in multiplex transcriptional reporter assays or as a normalizing transfection control for firefly luciferase assays. 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 also emits light from enzyme-independent oxidation, a process known as auto-luminescence. The auto-luminescence is enhanced by superoxide anion and peroxynitrite in cells and tissues.

This Renilla luciferase assay kit utilizes a special buffer formulation designed to yield reliable, linear measurements of Renilla luciferase activity with minimal auto-luminescence background and superior sensitivity. 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.

2. Products

Kit Components

Components	PR300002 (150 assays)	PR300007 (1000 assays)
5X Passive Lysis Buffer	10 mL	30 mL
Renilla Luciferase Assay Buffer 2.0	15 mL	100 mL
Aquaphile Coelenterazine	3 x 200 ug	1 x 4 mg

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 Protocols), additional 5X passive lysis buffer (Cat# PR300004) may be purchased separately.

Storage and Handling

Store the kit at -80°C. Renilla Luciferase Assay Buffer 2.0 is stable at -80°C for at least six 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.

Notice to purchaser

This product is for research use only. Use in and/or for diagnostics and therapeutics is strictly prohibited. By opening and using the product, the purchaser agrees to the following: The product may not be distributed, resold, modified for resale or used to manufacture commercial products without prior written approval from OriGene Technologies, Inc. If you do not agree to the above conditions, please return the

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UNOPENED product to OriGene Technologies, Inc. within ten (10) days of receipt for a full refund by contacting customer service at custsupport@origene.com.

3. Experimental protocol

Preparation of Cell Lysates

1. Prepare 1X Passive Lysis Buffer by adding 1 volume of 5X buffer to 4 volumes of dH2O and mixing well. 1X Passive Lysis Buffer may be stored at 4°C for up to one month.

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:

Wells/plate	Lysis buffer/well
6 well	500 uL
12 well	250 uL
24 well	100 uL
48 well	65 uL
96 well	20 uL

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.

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

Preparation of Renilla Working Solution

1. Thaw Renilla Luciferase Assay Buffer 2.0 at room temperature.

2. Prepare 2 mg/mL Aquaphile coelenterazine stock solution. For 200ug coelenterazine (from Cat# PR300002), add 100 uL water to the vial and mix. For 4mg coelenterazine (from Cat# PR300007), add 2 mL water to the vial and mix. Stock solutions of Aquaphile coelenterazine can be stored for up to 3 months at -20°C or below.

3. Prepare enough Renilla working solution to perform the desired number of assays (100 uL working solution per assay). Dilute Aquaphile coelenterazine (2 mg/mL) in Renilla Luciferase Assay Buffer 2.0 at a ratio of 1:50. For example, add 20 uL Aquaphile coelenterazine stock solution to 1 mL assay buffer.

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Note: 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.

Renilla Luciferase Assay

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

1. Set up luminometer with parameters recommended for your instrument for dual luciferase assay. We routinely use integration time of 1 second.

2. Add 20 uL of cell lysate into a reaction tube that is compatible with your luminometer.

3. Add 100 uL of Renilla working solution to the reaction tube and mix by pipetting or vortexing.

4. Immediately place tube in luminometer and record the Renilla luminescence measurement.

Determination of Assay Background

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.

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