Revised: November 4, 2016

Product Information

Renilla Luciferase Assay Kit 2.0

Catalog Number: 30082-T, 30082-1, 30082-2

Kit Contents

Component	30082-T 50 assays	30082-1 150 assays	30082-2 1000 assays
5X Passive Lysis Buffer	5 mL 99934	10 mL 99911*	30 mL 99912*
Renilla Luciferase Assay Buffer 2.0	5 mL 99816-5mL	15 mL 99816-15mL	100 mL 99816-100mL
Aquaphile™ Coelenterazine	1 x 200 ug 10126-200ug	3 x 200 ug 10126-200ug	1 x 4 mg 10126-4mg

* 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. no. 99912) 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.

Product Description

This kit is designed to measure *Renilla* luciferase activity in transfected cell reporter gene assays. *Renilla* luciferase has been used as a reporter gene for studying gene regulation and function in vitro and in vivo.[1,2] It commonly is used in multiplex transcriptional reporter assays or as a normalizing transfection control for firefly luciferase assays.[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 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. This assay kit utilizes a special buffer formulation designed to yield reliable, linear measurements of *Renilla* luciferase activity 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 R*enilla* Luciferase Assay Kit 2.0 features Biotium's Aquaphile™ Coelenterazine, a water soluble substrate that can be stored at -20°C with minimal evaporation, unlike methanol solutions of coelenterazine.

Biotium also offers the Firefly & *Renilla* Luciferase Single Tube Assay Kit, 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).

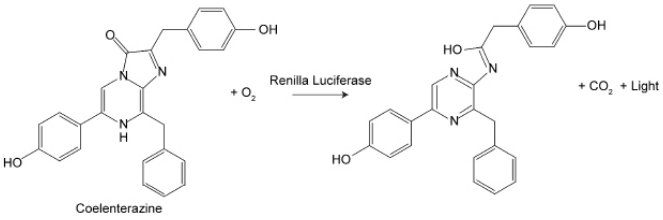

Figure 1. Bioluminescent reaction catalyzed by *Renilla* luciferase.

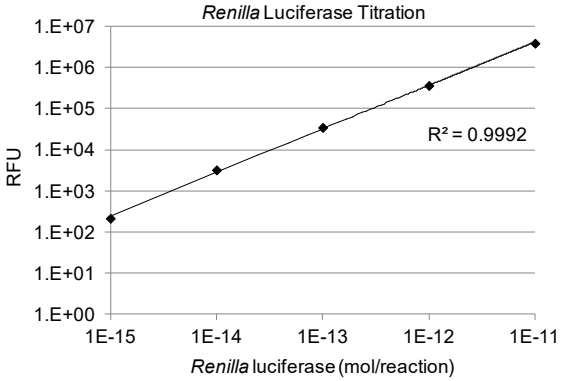

Figure 2. Titration of recombinant *Renilla* luciferase in the *Renilla* Luciferase Assay 2.0. *Renilla* luciferase (RayBiotech) was serially diluted in 1X Passive Lysis Buffer and measured in the assay. Luminescence was measured on a Promega Glomax® 20/20 single tube luminometer with integration time of 1 second. Background from reagents without enzyme added was subtracted from luminescence values.

References

1. Bhaumik S. et al. (2004) J Biomed Opt. 9, 578-86.
2. Matijasevic Z. et al. (2001) Carcinogenesis. 22, 661-4.
3. Nieuwenhuijsen BW. et al. (2004) J Biomol Screen. 8, 676-84.
4. Matthews, J.C., Hori, K. and Cormier, M.J. (1977) Biochemistry 16, 85–91.

Assay Protocols

Preparation of cell lysates

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.
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. Biotium offers mini cell scrapers (cat. no. 22003) for harvesting lysates from 96-, 24-, and 48-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.

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 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 -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. 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 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.

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 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 reaction.

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.

Related Products

Catalog number	Product
99912	5X Passive Lysis Buffer, 30 mL
22003	Mini Cell Scrapers, pack of 200
30085	Firefly Luciferase Assay Kit 2.0
30075	Firefly Luciferase Assay Kit (Lyophilized)
30081	Firefly & *Renilla* Luciferase Single Tube Assay Kit
30028	Steady-Luc™ Firefly HTS Assay Kit
30028L	Steady-Luc™ Firefly HTS Assay Kit, Lyophilized
30020	ATP-Glo™ Bioluminometric Cell Viability Assay
10126	Aquaphile™ Coelenterazine
10127	Aquaphile™ Coelenterazine h

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