

Renilla Luciferase Assay

Cell Based Assay

Cat. # SCT153

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NOT FOR USE IN DIAGNOSTIC PROCEDURES.
NOT FOR HUMAN OR ANIMAL CONSUMPTION.

pack size: 1 Kit

Store at -80°C



Data Sheet

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Background

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. 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.

The *Renilla* Luciferase Assay utilizes a special buffer formulation designed to yield reliable, linear measurements of *Renilla* luciferase activity with minimal autoluminescence 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.

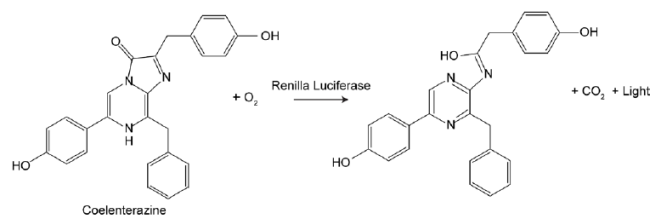


Figure 1. Assay principle. Bioluminescent reaction catalyzed by *Renilla* luciferase.

Storage

Store *Renilla* Luciferase Assay 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.

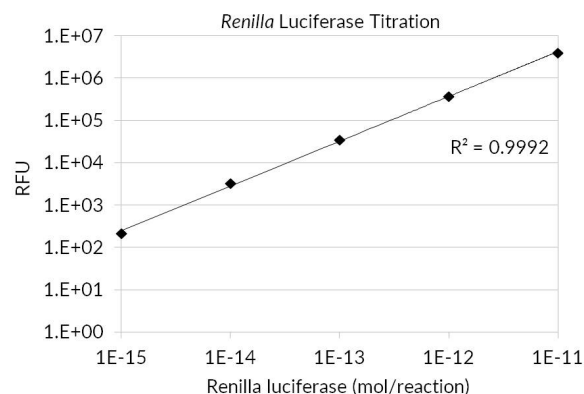


Figure 2. Titration of recombinant *Renilla* luciferase in the *Renilla* Luciferase Assay. Recombinant *Renilla* luciferase was serially diluted in 1X Passive Lysis Buffer with 1 mg/mL BSA and measured in the assay. Luminescence was measured on a Promega Glomax® 20/20 single tube luminometer with integration time of 1 second.

Kit Components

- 1) 5X Passive Lysis Buffer (CS224582): 30 mL
- 2) *Renilla* Luciferase Assay Buffer 2.0 (CS224583): 100 mL
- 3) Aquaphile™ Coelenterazine (CS224584): 1 X 4 mg

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Assay 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 µL
12-well	250 µL
24-well	100 µL
48-well	65 µL
96-well	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.

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. Add 2 mL water to the 4 mg 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 µL 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 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.

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 multiwell plate according to the instructions for your instrument.

1. Set up luminometer with parameters recommended for your instrument. 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 firefly working solution to the reaction tube and mix by pipetting or vortexing.
4. Immediately place tube in luminometer and record the firefly 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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