Firefly/*Renilla* Dual Luciferase Assay

Cell Based Assay

Cat. # SCT152

pack size: 1 Kit

Store at -80ºC

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

Data Sheet

Background

Firefly luciferase is widely used as a reporter for studying gene regulation and function, and for pharmaceutical screening. It is a very sensitive genetic reporter due to the absence of endogenous luciferase activity in mammalian cells or tissues. *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 Firefly/*Renilla* Dual Luciferase Assay allows measurement of both Firefly and *Renilla* luciferase activity in the same sample with high sensitivity and linearity. Firefly luciferase activity is measured first, then Renilla Luciferase Assay Buffer 2.0 is added to simultaneously quench firefly luciferase activity and measure *Renilla* luciferase activity. *Renilla* Luciferase Assay Buffer 2.0 quenches the firefly luciferase activity to the level of untransfected cells, allowing sequential measurement of firefly and Renilla luciferase activity in the same sample. This is a flash-type assay that requires luminescence to be measured immediately after adding the detection reagents to the luciferase sample. Firefly signal decays over the course of about 12 minutes, while Renilla signal decays over the course of about 2 minutes, although this may vary depending on enzyme levels.

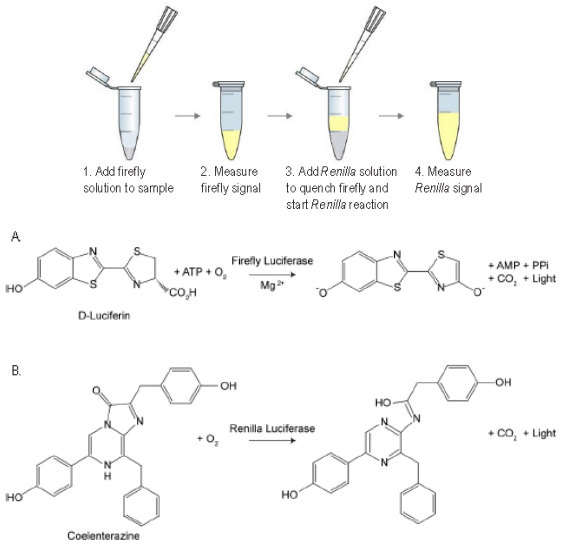

Figure 1. Bioluminescent reactions catalyzed by firefly luciferase and Renilla luciferase.

Kit Components

1) 1X Passive Luciferase Lysis Buffer 2.0 (CS224514): 15 mL

2) Firefly Luciferase Assay Buffer 2.0 (CS224591): 15 mL

3) D-Luciferin (CS224551): 3 X 1 mg

4) *Renilla* Luciferase Assay Buffer 2.0 (CS224580): 15 mL

5) Aquaphile™ Coelenterazine (CS224581): 3 X 200 µg

Storage

Store Firefly/*Renilla* Dual Luciferase Assay at -80°C. Firefly and *Renilla* Assay Buffers are stable at -80°C for at least six months from date of receipt. Other components are stable at -20°C or below for at least six months from date of receipt. Kit components and stock solutions of D-Luciferin and Aquaphile Coelenterazine in water are stable to at least 5 freeze/thaw cycles.

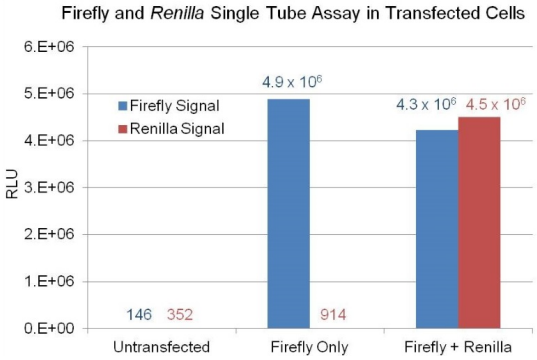

Figure 2. Example of Firefly & *Renilla* Luciferase detection using lysates from untransfected HeLa cells or cells transfected with either firefly luciferase alone (Firefly Only) or co-transfected with firefly and *Renilla* luciferases (Firefly + Renilla). In cells transfected with firefly only, the Renilla signal is the residual firefly luminescence after adding Renilla working solution to the reaction.

Rev.1.0/2018-01-29/SCT152/NJA

Assay Protocol

Preparation of Cell Lysates

1. 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

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. 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 Firefly Working Solution

1. Thaw Firefly Luciferase Assay Buffer 2.0 at room temperature.
2. Prepare 10 mg/mL D-luciferin stock solution. Add 100 µL water to the 1 mg vial and mix. The stock solution can be stored for at least 6 months at -20°C or below, and is stable to up to 5 freeze/thaw cycles.
3. Prepare enough firefly working solution to perform the desired number of assays (100 µL working solution per assay). Add D-luciferin (10 mg/mL) to assay buffer at a ratio of 1:50. For example, add 20 µL D-luciferin stock solution to 1 mL firefly assay buffer.

   Note: For best results, working solutions (assay buffer with substrate) should be prepared fresh before each use, and used within 3 hours of preparation. Firefly working solution activity decreases ~10% after 3 hours and ~25% after 5 hours at room temperature.

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 100 µL 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.

Firefly/*Renilla* Dual 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 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 firefly working solution to the reaction tube and mix by pipetting up and down several times.
   Note: Do not vortex the tube, which could cause the firefly reaction mix to coat the upper part of the tube and not effectively mix with the Renilla working solution in step 5.
4. Immediately place tube in luminometer and record the firefly luminescence measurement.
5. Add 100 uL of Renilla working solution to the same reaction tube and mix by pipetting or vortexing.
6. Immediately place tube in luminometer and record the Renilla luminescence measurement.
7. Discard the reaction tube, and proceed to the next reaction.

Note: Renilla working solution can be used to measure Renilla luciferase activity in the absence of firefly luciferase, but for direct comparison to samples with both Firefly and Renilla luciferases, you should first add firefly working solution before adding Renilla working solution so the final assay volume remains constant between samples. For determination of Renilla activity only, firefly working solution can be omitted.