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DATA SHEET

Version: 03
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1. Identification

Product name

Firefly & Renilla Dual Luciferase Single Tube Assay Kit

150 assays

Cat. No: **CA141**

2. Description

The Firefly & Renilla Dual Luciferase Assay Kit exploits the differing biochemical requirements for luminescence of the firefly (Photinus pyralis) and sea pansy (Renilla reniformis) luciferase proteins. The kit allows the sequential quantitative measurement of both luciferase activities in a single protein extract. Both, the firefly and Renilla luciferase proteins, have proven to be highly effective as gene reporters, because the assays are extremely sensitive, rapid, reproducible, and easy to perform.
Firefly luciferase has an apparent molecular weight of 62 kDa, which is active as a monomer and does not require subsequent processing for its activity. The enzyme catalyzes the oxidation of reduced luciferin in the presence of ATP-Mg^{2+} and oxygen to generate CO_2, AMP, PPi, oxyluciferin, and produces a flash of light that is proportional to the quantity of luciferase in the reaction mixture. Renilla luciferase (Rluc) is a 36- kDa monomeric protein that is glycosylated in its natural host; however, this posttranslational modification is not required for its activity. The luminescence generated by Renilla luciferase utilizes O_2 and coelenterazine. The dissimilarity in the substrates for the two luciferases makes it possible to selectively distinguish between the luminescent reactions for each enzyme. The luminescence of the firefly luciferase can be measured by addition of the luciferin reagent, and this reaction is subsequently quenched while simultaneously activating the luminescence of the Renilla luciferase. Thus, one can sequentially measure the luminescence of both reporters in a single reaction tube.

3. Composition

Item	Quantity
1X Cell Lysis Buffer*	15 mL
Fluc Assay Buffer	15 mL
Rluc Assay Buffer	15 mL
D-Luciferin, potassium salt	3 x 1 mg
Coelenterazine (lyophilized)	3 x 200 µg

*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 plates.

4. Storage specifications

Fluc and Rluc Assay Buffers are stable at -80 °C. Other components are stable at -20°.
Kit components are stable to at least 5 freeze/thaw cycles.

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5. Assay Procedure

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 Cell lysis buffer using the volume recommended below for each type of well:

Wells/plate	Lysis buffer/well
6 Wells	500 µL
12 Wells	250 µL
24 Wells	100 µL
48 Wells	65 µL
96 Wells	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 Cell 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 Cell 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 Fluc Assay Buffer at room temperature.
2. Prepare 10 mg/mL D-luciferin stock solution**: Add 100 uL water to the D-Luciferin, potassium salt (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 uL working solution per assay). Dilute D-luciferin (10 mg/mL) in assay buffer at a ratio of 1:50. For example, add 20 uL D-luciferin stock solution to 1 mL Fluc 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 RLuc Luciferase Assay Buffer at room temperature.
2. Prepare 2 mg/mL coelenterazine stock solution**: Add 100 uL water to the Coelenterazine (lyophilized) (200ug) vial and mix.** The stock solution can be stored for up to 3 months at -20°C or below and is stable to up to 5 freeze/thaw cycles.
3. Prepare enough Renilla working solution to perform the desired number of assays (100 uL working solution per assay). Dilute coelenterazine (2 mg/mL) in Rluc Assay Buffer at a ratio of 1:50. For example, add 20 uL coelenterazine stock solution to 1 mL 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. Renilla working solution activity is stable for up to 3 hours, but background increases up to 60% after 5 hours at room temperature.*

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Firefly & 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 one or both working solutions into each luminometer tube or well of a multiwall 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.*

6. Further information

Product Use Limitations This product is developed, designed and sold exclusively only for research purposes use. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

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