Product Data Sheet

Product Name: Dual Luciferase Reporter Gene Assay Kit
Cat. No.: GK10029

Components

Components	Quantity	Storage
1X Passive Luciferase Lysis Buffer	10ml	-20°C Protect from light 1 year
Firefly Luciferase Assay Buffer	10ml	-20°C Protect from light 1 year
D-Luciferin	2mg	-20°C Protect from light 1 year
Renilla Luciferase Assay Buffer	10mL	-20°C Protect from light 1 year
Coelenterazine	400ug	-20°C Protect from light 1 year

Protocol

Preparation of cell lysates

Note: 1X Passive Lysis Buffer is ready to use without dilution.

1.1 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 2.0 using the volume recommended below for each type of well:

Cell Culture Plate	96-well plates	48-well plates	24-well plates	12-well plates	6-well plates
Lysis Buffer (A) uL/per well	20uL	65uL	100uL	250uL	500uL

1.2 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.

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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.

Note: 1X Passive Lysis Buffer contains protein stabilizers that may affect results of protein quantitation assays.

1.3 Transfer the lysate to a tube or vial. Optional: the lysate can be cleared by centrifugation for 30 seconds at top speed in a refrigerated microcentrifuge and transferred into a new tube. Place at 4°C until ready to assay. Lysates can be stored at -20°C or -80°C for up to two weeks.

Preparation of Firefly Working Solution

2.1 Thaw Firefly Luciferase Assay Buffer 2.0 at room temperature.

2.2 Prepare 10 mg/mL D-luciferin stock solution. For component C (2mg), add 200 uL water to the 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.

2.3 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 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

3.1 Thaw Renilla Luciferase Assay Buffer at room temperature.

3.2 Prepare 2 mg/mLcoelenterazine stock solution. For component E-400ug, add 200 uL EtOH to the 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.

3.3 Prepare enough Renilla working solution to perform the desired number of assays (100 uL working solution per assay). Dilute coelenterazine (2 mg/mL) in Renilla Luciferase 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.

Firefly & Renilla Luciferase Single Tube 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 multiwell plate according to the instructions for your instrument.

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

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

4.3 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.

4.4 Immediately place tube in luminometer and record the firefly luminescence measurement.

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

4.6 Immediately place tube in luminometer and record the Renilla luminescence measurement. 4.7 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.

NOTE:

Always wear lab coats, gloves and goggles when working with our products although they are low-risk chemicals for R&D only.

Background

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Dual Luciferase Reporter Gene Assay Kit

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

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