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.

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.

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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.

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

Background

双萤光素酶报告基因检测试剂盒(Dual Luciferase Reporter Gene Assay Kit)，是先以萤光素(luciferin)为底物来检测萤火虫萤光素酶(Firefly luciferase)，后以肠腔素(coelenterazine)为底物来检测海肾萤光素酶(Renilla luciferase)，并且在后续加入海肾萤光素酶底物时，同时加入抑制萤火虫荧光素酶催化 luciferin 发光的物质，使后续检测仅仅检测到海肾萤光素酶的活性，实现双萤光素酶报告基因检测。
萤火虫萤光素酶是一种分子量约为 61kD 的蛋白，在 ATP 、镁离子和氧气存在的条件下，可以催化 luciferin 氧化成 oxyluciferin，在luciferin 氧化的过程中，会发出生物萤光(bioluminescence)。海肾萤光素酶是一种分子量约为 36kD 的蛋白，在氧气存在的条件下，可以催化 coelenterazine 氧化成 coelenteramide，在 coelenterazine 氧化的过程中也会发出生物萤光。生物萤光可以通过化学发光仪(luminometer)或 液闪测定仪进行测定。本试剂盒的检测原理参考图 1.
双萤光素酶报告基因检测试剂盒为检测基因的表达量提供有效的手段，在 DLR 检测中，萤火虫萤光素酶（Firefly luciferase）和海肾萤光素 酶（Renilla luciferase） 的活性可在单个样品中依次检测。先以萤光素（Luciferin）为底物来检测萤火虫萤光素酶的活性，然后加入抑制萤火 虫萤光素酶催化的物质，同时加入腔肠素（Coelenterazine）检测海肾萤光素酶的活性，实现双萤光素酶报告基因检测。通过萤光素酶和其底 物这一生物发光体系，可以非常灵敏、高效地检测基因的表达。通常把感兴趣基因的转录调控元件或 5' 启动子区克隆在Luciferase 的上游，或把 3'-UTR 区克隆在Luciferase 的下游，构建成报告基因（Reporter gene）质粒，然后转染细胞，用适当药物等处理细胞后裂解细胞，通过检测萤光素酶活性的高低来判断药物处理等对目的基因的转录调控作用。海肾萤光素酶更多地被用作检测转染效率的内参，以消除细胞数量 和转染效率的差异。
萤火虫萤光素酶催化 luciferin 发光的最强发光波长为 560nm。海肾萤光素酶催化 coelenterazine 发光的最强发光波长为 465nm。
本试剂盒的光信号可以通过化学发光仪、酶标仪或液闪测定仪进行测定。该试剂盒具有检测迅速、灵敏度高、检测范围广，无细胞内源活性干扰等特点。

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

保存条件：

最好在-20℃保存。将 C 组分溶解到 B 组分后，该混合液不可反复冻融，建议进行小批量分装，并于-20℃(最长可储存 6 个月)条件下储存。 Renilla Luciferase Assay solution（ D+E）应新鲜配制，当天使用 。