Double-Luciferase Reporter Assay Kit

Catalogue No.:abx098134

Double-Luciferase Reporter Assay Kit provides an efficient means of detecting the activities of firefly (photinus pyralis) and renilla (sea pansy) luciferases, using luciferin and coelenterazine as substrates respectively.

Kit contents:

Component	50 rxns	200 rxns
Luciferase Reaction Buffer 1	5 ml	20 ml
Luciferase Reaction Buffer 2	5 ml	20 ml
Luciferase Reaction Substrate 1	1 vial	4 vials
Luciferase Reaction Substrate 2 (50X)	100 µl	400 µl
Cell Lysis Buffer (5X)	5 ml	20 ml

Reagents required but not provided:

- PBS (1X)
- Nuclease-Free Water

Target: Double-Luciferase Reporter Assay Kit

Storage: Store at -20 °C. Once prepared (see Assay Procedure), store the Luciferase Reaction Reagents 1 and 2 in the dark at -20 °C for up to one month or -70 °C for up to 1 year.

Note: THIS PRODUCT IS FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC, THERAPEUTIC OR COSMETIC PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION.
This product is shipped with dry ice.

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Directions for use:

Assay Procedure

1. Bring the Luciferase Reaction Buffer 1 and Luciferase Reaction Buffer 2 to room temperature. If precipitates are observed in Luciferase Reacton Buffer 2, mix well and/or heat in a 37 °C water bath until they are dissolved.
2. Prepare the Luciferase Reaction Reagent 1 by adding 5 ml of Luciferase Reaction Buffer 1 into 1 vial of Luciferase Reaction Substrate 1. **Mix well and store in the dark at -20 °C for up to 1 month, or at -70 °C for up to 1 year. Avoid repeated freeze/thaw cycles.**
3. Prepare the Luciferase Reaction Reagent 2 by adding Luciferase Reaction Substrate 2 to Luciferase Reaction Buffer 2 at a ratio of 1:49 (e.g. add 50 µl of Luciferase Reaction Substrate 2 to 2.45 ml of Luciferase Reaction Buffer 2). **Mix well and store in the dark at -20 °C for up to 1 month, or at -70 °C for up to 1 year. Avoid repeated freeze/thaw cycles.**
4. Prepare the 1X Cell Lysis Buffer by adding 5X Cell Lysis Buffer to Nuclease-Free Water at a ratio of 1:4 (e.g. add 1 ml of 5X Cell Lysis Buffer to 4 ml of Nuclease-Free Water).
5. Remove the culture medium. Carefully rinse twice with 1X PBS and add an appropriate volume of 1x Cell Lysis Buffer according to the table below. Fully lyse cells at room temperature for 10 minutes. Scrape the cells into a microcentrifuge tube, then centrifuge at 12,000 × g for 10 minutes. Take the supernatant, which contains the cell lysate.

Cell Culture Plate Type	Lysis Buffer per Well
6-well	500 µl
12-well	250 µl
24-well	100 µl
48-well	60 µl
96-well	20 µl

6. Bring the Luciferase Reaction Reagent 1 and Luciferase Reaction Reagent 2 to room temperature.
7. Add 100 µl of Luciferase Reaction Reagent 1 (equilibrated to room temperature) into a microcentrifuge tube or well in an opaque 96-well plate. Add 20 µl of cell lysate and mix thoroughly. Measure the luminescence using a luminometer to measure the firefly luciferase activity.
8. Add 100 µl of Luciferase Reaction Reagent 2 (equilibrated to room temperature) into a microcentrifuge tube or well in an opaque 96-well plate. Add 20 µl of cell lysate and mix thoroughly. Measure the luminescence using a luminometer to measure the renilla luciferase activity.

Notes:

• It is recommended to add samples and reagents using a multichannel pipette in steps 7 and 8, when measuring a large number of samples.
• Luciferase Reaction Reagents 1 and 2 are easily oxidized. It is recommended to use Luciferase Reaction Reagents 1 and 2 immediately after they are equilibrated to room temperature.

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