Double-Luciferase Reporter Assay Kit

Catalog#JKR23008-50T

Instruction Manual

Sufficient reagents for 50T Double-Luciferase Reporter Assay per kit.
Store at -20°C

Table of Contents

1. Storage

Store the kit at -20°C. Cell Lysis Buffer can be stored at -20°C for one year. Prepared luciferase reaction reagents (Luciferase Reaction Reagent and Luciferase Reaction Reagent II) should be aliquoted and stored protected from light; they can be stored at -70°C for one year or at -20°C for one month.

2. Product Description

Firefly luciferase and Renilla luciferase can catalyze the oxidation of luciferin or coelenterazine to form oxyluciferin or coelenteramide, respectively, generating bioluminescence in the process. The Double-Luciferase Reporter Assay Kit first detects the activity of the firefly luciferase reporter gene using luciferin as a substrate, then quenches this luminescent reaction and detects the activity of the Renilla luciferase reporter gene using coelenterazine as a substrate. It features rapid detection, high sensitivity, a broad detection range, and no interference from endogenous cellular activity.

3. Kit Components

Component	JKR23008 (50 rxns)	Storage Temperature
Luciferase Reaction Buffer	5 mL	-20°C
Luciferase Reaction Substrate (50X)	100 μL	-20°C
Luciferase Reaction Buffer II	5 mL	-20°C
Luciferase Reaction Substrate II (50X)	100 μL	-20°C
Cell Lysis Buffer	25 mL	-20°C

4.Operating Steps

Self-supplied

Product Name
PBS (1X): Formula see appendix
Small steel beads or mortar and pestle

4.1 Reagent Preparation

Remove the Luciferase Reaction Buffer and Luciferase Reaction Buffer II from -20°C and allow them to reach room temperature, ensuring all components are fully dissolved. (Note:Precipitation in Luciferase Reaction Buffer II is normal; it can be dissolved by incubating in a 37°C water bath for about 20 min, followed by thorough vortexing before use.)

4.1.1 Luciferase Reaction Reagent

Mix Luciferase Reaction Substrate with Luciferase Reaction Buffer at a 1:49 ratio, and assay the lysate within 30 min (preferably prepared fresh). Remaining reagent should be stored protected from light at -20°C or -70°C.

4.1.2 Luciferase Reaction Reagent II

Mix Luciferase Reaction Substrate II with Luciferase Reaction Buffer II at a 1:49 ratio, and assay the lysate within 30 min (preferably prepared fresh). Remaining reagent should be stored protected from light at -20°C or -70°C.

4.2 Sample Processing

a.Animal Cells

1) Adherent cells: Remove the cell culture medium, gently rinse twice with 1X PBS, add an appropriate amount of Cell Lysis Buffer, fully lyse at room temperature for 10 min, then scrape the cells into a 1.5 mL centrifuge tube.
2) Suspension cells: Collect the cells, centrifuge at 300xg for 5 min, and remove the medium. Add an appropriate amount of Cell Lysis Buffer, pipette to mix, and fully lyse at room temperature for 10 min.

Cell Culture Plate	Cell Lysis Buffer/Well
6-well	500 μL
12-well	300 μL
24-well	150 μL
48-well	60 μL
96-well	20 μL

After lysis is complete, centrifuge at 2-8°C and 12,000xg for 10min, then collect the supernatant for testing.

b.Plant leaves (using tobacco leaves as an example)

1) Place 3-4 tobacco leaves with a diameter of 6-8 mm into a 2 mL EP tube, add 3-4 pre-chilled small steel beads and an appropriate amount of liquid nitrogen, then grind and crush using a homogenizer; or place 3-4 tobacco leaves with a diameter of 6-8 mm into a mortar, add an appropriate amount of liquid nitrogen, and grind and crush with a pestle.
2) After grinding to a powder, add 300 μL of Cell Lysis Buffer (100μL lysis buffer per leaf). Mix by pipetting, and fully lyse at room temperature for 5 min.
3) Centrifuge at 2-8°C, 12,000 xg for 2 min, and collect the supernatant for testing.

c.Protoplasts

1) Collect protoplasts, count, centrifuge at 300xg for 5 min, and remove the supernatant.
2) Add lysis buffer according to 10^5 protoplasts /100 μL Cell Lysis Buffer, mix by pipetting, and fully lyse at room temperature for 10 min.
3) Centrifuge at 2-8°C, 12,000 xg for 2 min, and collect the supernatant for testing.

4.3 Fluorescence Detection

Aspirate 10–20 μL of lysate into an opaque 96-well plate, add 100 μL of Luciferase Reaction Reagent (equilibrated to room temperature) into each well. Mix thoroughly by gentle horizontal shaking. Measure luminescence using a luminometer with detection at

350–700 nm and an integration time of 1 sec to assess firefly luciferase reporter activity. (If signal saturation occurs, reduce the volume of lysate used for the assay). Then, aspirate 100 μL of Luciferase Reaction

Reagent II equilibrated to room temperature, add it to the reaction tube or plate, mix by horizontal shaking, and measure luminescence at 380–780 nm in a luminometer with a detection time of 1 second to assess Renilla luciferase reporter gene activity. Result calculation: firefly luminescence reading / Renilla luminescence reading.

5. Precautions

- Some precipitation may occur during the dissolution of Luciferase Reaction Buffer II; before use, ensure it is fully dissolved by vortexing thoroughly or placing it in a 37°C water bath.
- Luciferase Reaction Reagent and Luciferase Reaction Reagent II should be equilibrated to room temperature prior to reaction.
- To ensure accurate and reliable experimental data, it is recommended to use a multichannel pipette for adding Luciferase Reaction Reagent and Luciferase Reaction Reagent II when measuring a large number of samples, and always check that each channel aspirates the same volume.
- Both Luciferase Reaction Reagent and Luciferase Reaction Reagent II are prone to oxidation; please plan experiments accordingly to avoid prolonged exposure of thawed samples at room temperature.

6. Appendix

PBS preparation method: Weigh 8g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4 , and 0.24 g KH_2PO_4 , dissolve in 800 mL distilled water, adjust the solution pH to 7.4 using HCl, then add distilled water to a final volume of 1 L. Sterilize by autoclaving at 15 lbf/in2 (1034×105Pa) for at least 20 min, and store at room temperature or in a 4°C refrigerator.

WUHAN GENECREATE BIOLOGICAL ENGINEERING CO.,LTD.

Address： Wuhan Shengzhiyuan Biotechnology Innovation Industrial Park,
North Shendun 5 th Road, Jiangxia District, Wuhan City, Hubei Province.
Phone: 027-87960366
Email: marketing@genecreate.com
Website: www.genecreate.cn