Customer service: service@abbkine.com
Technical support: support@abbkine.com
Website: https://www.abbkine.com

Dual Luciferase Reporter Gene Assay Kit

Cat #: KTA8010 Size: 100 T/1000 T

	Dual Luciferase Reporter Gene Assay Kit		
REF	**Cat #:** KTA8010	LOT	**Lot #:** Refer to product label
	Applicable samples: Cells, Protoplasts and Plant leaves		
	Storage: Stored at -20℃ for 12 months, protected from light		

Assay Principle

In the presence of ATP, magnesium ions and oxygen, Firefly luciferase can catalyze firefly luciferin to Oxyluciferin, which generate light signals during the oxidation of luciferin. In the presence of oxygen, Renilla luciferase can catalyze the oxidation of coelenterazine to coelenteramide, and light signals are also generated during the oxidation of coelenterine. Through the Bioluminescence system of Luciferase and its substrate, gene expression can be detected very sensitively and efficiently. Usually, the Transcriptional regulation element or 5'-promoter region of the gene of interest is cloned upstream of Luciferase, or the 3'-UTR region is cloned downstream of Luciferase to construct a Reporter gene plasmid, and then transfect the cell, treat the cell with appropriate drugs, and then Lytic cells. The Transcriptional regulation effect of drug treatment on the target gene is judged by detecting the activity of Luciferase. Renilla luciferase is more used as an internal reference for detecting transfection efficiency to eliminate the difference between cell number and transfection efficiency. Dual Luciferase Reporter Gene Assay Kit first detects the activity of Firefly luciferase with the Luciferin as the substrate, then adds substances that inhibit the catalysis of Firefly luciferase, and at the same time adds Coelenterazine to detect the activity of Renilla luciferase, so as to realize the detection of dual Luciferase Reporter gene. This kit has the characteristics of rapid detection, high sensitivity, wide detection range and no interference from intracellular activity.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	100 T	1000 T	
Lysis Buffer (5×)	10 mL	50 mL×2	-20℃
Firefly Luciferase Assay Buffer	10 mL	100 mL	-20℃, protected from light
Firefly Luciferase Substrate	1	1	-20℃, protected from light
Renilla Luciferase Assay Buffer	10 mL	100 mL	4℃
Renilla Luciferase Substrate (50×)	200 μL	1 mL×2	-20℃, protected from light

Materials Required but Not Supplied

- Cell culture plate, precision pipettes, disposable pipette tips
- Deionized water , PBS, deionized water
- Refrigerated centrifuge, 96-well black plate or 96-well white plate
- Luminometer or multimode reader

Reagent Preparation

Lysis Buffer: Prepare before use, Lysis Buffer (5×) contains insoluble matter, shake well before use it. Dilute Lysis Buffer (5×) 5 times with deionized water to obtain Lysis Buffer, Store at -20℃.

Firefly Luciferase Solution: Prepare before use, dissolve the Firefly Luciferase Substrate with the Firefly Luciferase Assay Buffer, then transfer all of it to the bottle containing the Firefly Luciferase Assay Buffer, mix well, and then pack according to usage requirements and stored at -80℃, protected from light.

Note: Firefly Luciferase Solution cannot be repeatedly freeze-thawed. If it is used less in a single experiment, it is recommended to pack it into small sizes according to the amount used in a single experiment. Store at -20℃, protected from light, recommended for use within 3 months, store at -80℃, protected from light, effective for 12 months.

Renilla Luciferase Solution: Prepare before use, according to the actual usage, mix an appropriate amount of Renilla Luciferase Assay Buffer and Renilla Luciferase Substrate (50×) in a ratio of 50:1.

Note: Renilla Luciferase Solution needs to be ready each time, Renilla Luciferase Substrate was dissolved in anhydrous ethanol, it is necessary to centrifuge to the bottom of the tube immediately before use, and carefully measure the volume of the solution in the tube. If there is a significant decrease in liquid volume, please add anhydrous ethanol to replenish the volume and store it.

Assay Procedure

1. Cell lysis:

(1) Cells

a. Cells were cultured in appropriate well plates, then transfected and treated with appropriate methods.

b. Remove the medium, gently wash the cells with PBS twice (do this for the adherent cells, suspended cells can be directly centrifuged to collect cells), discard PBS, and add the Lysis Buffer as follows.

Reagent	96-well Plate	48-well Plate	24-well Plate	12-well Plate	6-well Plate
Lysis Buffer (μL)	100	150	200	300	500

Note: Shake Lysis Buffer well before use. If the expression level of luciferase is low, the amount of Lysis Buffer can be appropriately reduced, such as adding 100 μL to 24-well plate per well and 200 μL to 6-well plate per well.

c. Place the cells on a shaking table and shake for 5-10 min to fully lysate the cells.

d. Centrifuge the cell lysate at 10,000 rpm for 2 min, and take the supernatant for detection.

(2) Protoplasts (for reference only)

a. Prepare protoplasts and transform the corresponding plasmids into protoplasts.

b. Collect 2×10^5 protoplasts by centrifugation after 16-24 h, add 200 μL Lysis Buffer.

c. Incubate at room temperature for 5-10 min to fully lysate the protoplasts.

d. Centrifuge the protoplast lysate at 10,000 rpm for 2 min, and take the supernatant for detection.

(3) Plant leaves (for reference only)

a. Inject Agrobacterium containing the corresponding plasmid into plant leaves.

b. Take approximately 1 cm^2 of leaves 2 days after injection and add 500 μL Lysis Buffer, grind with homogenizer and centrifuge at10,000 rpm for 2 min, take the supernatant for detection.

Note: After cell lysis, luciferase activity can be immediately detected, and it can also be frozen for further testing if

necessary. The frozen sample should be melted to room temperature before testing.

2. Carefully aspirate 20-100 μL (If the sample is sufficient, please add 100 μL. If the sample is insufficient, the usage of the sample can be appropriately reduced, but the usage of each batch of samples should be consistent.) of cell lysis supernatant into a detection tube or 96-well black/white plate plate. Then add 100 μL of Firefly Luciferase Solution equilibrated to room temperature into the tube or plate, mix quickly, and immediately detect the Firefly Luciferase reporter gene activity by using luminometer or multimode reader.

3. Add 100 μL freshly prepared Renilla Luciferase Solution to the above reaction solution, mix quickly, and then immediately detect the Renilla Luciferase reporter gene activity by using luminometer or multimode reader.

Precautions

1. Due to the influence of temperature on enzyme reactions, both the sample and solution need to be equilibrated to room temperature before testing.
2. To achieve the best measurement effect, when using a single tube luminometer for measurement, the time from mixing each sample and solution to before measurement should be controlled as consistent as possible. When using a multimode reade with chemiluminescence detection function, it is advisable to add all samples first, and then add **Firefly Luciferase Solution**.
3. During testing, a 96-well black or white plate should be used to prevent interference from adjacent wells.
4. The maximum wavelength of bioluminescence catalyzed by firefly luciferase is 560 nm, and the maximum wavelength of bioluminescence catalyzed by renilla luciferase is 480 nm.
5. Renilla Luciferase Substrate is volatile and should be sealed for storage.

Strawberry moment: In addition to Dual Luciferase Reporter Gene Assay Kit (KTA8010), Abbkine also offers SuperKine™ Lipo3.0 Efficient Transfection Reagent (BMU111-EN) and other cell state assay kits, such as Sensitivity Cell Counting Kit-8 (BMU106-EN), EdU Cell Proliferation Image Kit (KTA2030/KTA2031) ,Apoptosis Detection kit (KTA0002), One-step TUNEL Apoptosis Assay Kit (KTA2010/KTA2011), etc. Scan the QR code on the right and follow the Abbkine official account to learn more about Abbkine products.

FAQ

Question	Answer
Does this product require the sensitivity of the microplate reader? Glow or astigmatism?	This product does not require sensitivity, using glow detection.
Is the renilla luciferase assay reagent added to the Wells of the firefly assay reagent after the value is detected by the firefly fluorescent reagent? Or add the renilla luciferase assay to a new well with cell lysates only?	Assays were performed in the same well, that is, firefly luciferase activity was detected at first, and then renilla luciferase activity.
If I only want to measure the renilla luciferin level, can I skip the luciferase step and directly add renilla luciferin working solution after lysis?	You still need to add the Firefly Luciferase Solution first.
What is the role of the renilla luciferase assay substrate?	The substrate not only inhibits the firefly luciferase activity, but also detects the renilla luciferase activity.
Does the step in the 96-well plate, after sample lysis, need to be done on ice?	No need to operate on ice, it's recommended to operate at room temperature.

Recommended Products

Catalog No.	Product Name
BMU111-EN	SuperKine™ Lipo3.0 Efficient Transfection Reagent
BMU106-EN	SuperKine™ Maximum Sensitivity Cell Counting Kit-8 (CCK-8)
KTA2030	EdU Cell Proliferation Image Kit (Green Fluorescence)
KTA2010	One-step TUNEL Apoptosis Assay Kit (Green Fluorescence)
KTA0002	Annexin V-AbFluor™ 488/PI Apoptosis Detection kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.