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Luciferase Reporter Gene Assay Kit

Cat #: KTA8011 Size: 100 T/1000 T

	Luciferase Reporter Gene Assay Kit		
REF	**Cat #:** KTA8011	LOT	**Lot #:** Refer to product label
	Applicable samples: Cells		
	Storage: Stored at -20℃ for 12 months, protected from light		

Assay Principle

In the presence of ATP, magnesium ions and oxygen, Luciferase (also known as Firefly Luciferase) can catalyze firefly luciferin to Oxyluciferin, which generate light signals during the oxidation of luciferin. 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. Firefly Luciferase Reporter Gene Assay Kit detects the activity of Firefly luciferase with the Luciferin as the substrate, this kit has the characteristics of rapid detection, high sensitivity and wide detection range.

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

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.

Assay Procedure

1. Cells were cultured in appropriate well plates, then transfected and treated with appropriate methods.
2. Cell lysis:

(1) 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.

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

(3) Centrifuge the cell lysate at 10,000 rpm for 2 min, and 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.

3. 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.

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.

Recommended Products

Catalog No.	Product Name
KTA8010	Dual Luciferase Reporter Gene Assay Kit
BMC1041	Luminescent Mycoplasma Detection Kit
PRP3004	Luciferase firefly

Disclaimer

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