TransDetect® Single-Luciferase (Renilla) Reporter Assay Kit

Please read the data sheet carefully prior to use.

Cat. No. FR111

Version No. Version 2.0

Storage: The kit stored at -20°C for one year. Cell Lysis Buffer can be stored at -20°C for one year. Prepared Luciferase Reaction Reagent II should be stored in aliquots in dark at -20°C for one month or at -70°C for one year.

Description

Renilla luciferase catalyzes the oxidation of coelenterazine to form coelenteramide, and produces bioluminescence in the process. *TransDetect*® Single-Luciferase (*Renilla*) Reporter Assay Kit uses coelenterazine as a substrate to detect the activity of *Renilla* luciferase reporter gene. It has the characteristics of rapid detection, high sensitivity, wide detection range, and no interference of endogenous activity of cells.

Kit Contents

Component	FR111-01-V2 (50 rxns)	FR111-02-V2 (200 rxns)
Luciferase Reaction Buffer II	5 ml	20 ml
Luciferase Reaction Substrate II (50×)	100 µl	400 µl
Cell Lysis Buffer (5×)	5 ml	20 ml

Procedures

Self-prepared

Product Name	Catalogue
PBS(1×)	TransGen, Cat. FG701-01
Nuclease-free Water	TransGen, Cat. GI101-01

1. Reagent Preparation

(1) Luciferase Reaction Reagent II

Take out Luciferase Reaction Buffer II from -20°C and equilibrate to room temperature to ensure that all components are completely dissolved (Note: It is normal for Luciferase Reaction Buffer II to precipitate, and it can be used after sufficient shaking to dissolve). Mix Luciferase Reaction Substrate II with Luciferase Reaction Buffer II at a ratio of 1:49, and store in the dark after aliquoting to avoid repeated freezing and thawing.

(2) 1×Cell Lysis Buffer

Mix 5×Cell Lysis Buffer with Nuclease-free Water at a ratio of 1:4.

2. Lyse Cells

Remove the cell culture medium. Carefully rinse twice with 1×PBS, and add an appropriate amount of 1×Cell Lysis Buffer. Fully lyse at room temperature for 10 minutes. Scrape the cells into a 1.5 ml microcentrifuge tube, and centrifuge at 12,000×g at 2-8°C for 10 minutes. Take the supernatant (cell lysate) for use.

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

3. Fluorescence Detection

Add 100 µl of Luciferase Reaction Reagent II equilibrated to room temperature into a 1.5 ml microcentrifuge tube or opaque 96-well plate. Carefully pipette 20 µl of cell lysate into the reaction tube or plate, and shake horizontally to mix. The activity of the Renilla luciferase reporter gene was detected in a luminometer.

Notes

- Luciferase Reaction Buffer II may be partially precipitated during the dissolution process. Before use, it should be fully shaken or placed in a 37°C water bath to ensure that it is completely dissolved before use.
- Equilibrate to room temperature before Luciferase Reaction Reagent II.
- To ensure the accuracy and reliability of the experimental data, it is recommended to add Luciferase Reaction Reagent II with the multichannel pipette when measuring a large number of samples. During use, be sure to pay attention to whether the liquid absorbed by each channel of the pipette is consistent.
- Luciferase Reaction Reagent II is prone to oxidation reaction. Please arrange the experiment reasonably to avoid long-term storage of samples at room temperature after thawing.