

# TransDetect® Double-Luciferase Reporter Assay Kit

Please read the data sheet carefully prior to use.

Cat. No. FR201

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 should be stored in aliquots in dark at -20°C for one month or at -70°C for one year.

## Description

Firefly luciferase and Renilla luciferase can catalyze the oxidation of luciferin or coelenterazine to form oxyluciferin or coelenteramide, respectively, and produce bioluminescence in the process.

TransDetect® Double-Luciferase Reporter Assay Kit first uses luciferin as a substrate to detect the activity of the firefly luciferase reporter gene. Then, while quenching the fluorescence reaction, the activity of the Renilla luciferase reporter gene was detected by using coelenterazine as a substrate. It has the characteristics of rapid detection, high sensitivity, wide detection range, and no interference with the endogenous activity of cells.

## Kit Contents

Component	FR201-01-V2 (50 rxns)	FR201-02-V2 (200 rxns)
Luciferase Reaction Buffer	5 ml	20 ml
Luciferase Reaction Substrate (Lyophilized)	1 vial	4 vials
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

Take out Luciferase Reaction Buffer and 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).

#### (1) Luciferase Reaction Reagent

Use Luciferase Reaction Buffer to fully dissolve the lyophilized Luciferase Reaction Substrate (5 ml Buffer + 1 Vial Substrate), and store in the dark after aliquoting to avoid repeated freezing and thawing.

#### (2) Luciferase Reaction Reagent II

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.

#### (3) 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 $\mu$ l
12-well	250 $\mu$ l
24-well	100 $\mu$ l
48-well	60 $\mu$ l
96-well	20 $\mu$ l

### 3. Fluorescence Detection

Add 100  $\mu$ l of Luciferase Reaction Reagent equilibrated to room temperature into a 1.5 ml microcentrifuge tube or opaque 96-well plate. Carefully pipette 20  $\mu$ l of cell lysate into the reaction tube or plate, and shake horizontally to mix. The activity of the firefly luciferase reporter gene was detected in a luminometer. Then add 100  $\mu$ l of Luciferase Reaction Reagent II equilibrated to room temperature into a 1.5 ml microcentrifuge tube or opaque 96-well plate. Carefully pipette 20  $\mu$ l 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 and Luciferase Reaction Reagent II.
- To ensure the accuracy and reliability of the experimental data, it is recommended to add Luciferase Reaction Reagent and 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.
- Both Luciferase Reaction Reagent and Luciferase Reaction Reagent II are prone to oxidation reactions. Please arrange the experiment reasonably to avoid long-term storage of samples at room temperature after thawing.

**For research use only, not for clinical diagnosis.**

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