TransDetect® Double-Luciferase Reporter Assay Kit

Please read the data sheet carefully prior to use.

Cat. No. FR201

Version No. Version 2.1

Storage: The kit should be stored at -18°C or below. Cell Lysis Buffer can be stored at -18°C or below for one year. The prepared Luciferase Reaction Reagent and Luciferase Reaction Reagent II should be aliquoted and stored protected from light, stable for up to one year at -70 °C or below, and for up to one month at -18 °C or below.

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 (50 rxns)	FR201-02 (200 rxns)	FR201-03 (1000 rxns)
Luciferase Reaction Buffer	5 ml	20 ml	100 ml
Luciferase Reaction Substrate (Lyophilized)	1 vial	4 vials	1 vial
Luciferase Reaction Buffer II	5 ml	20 ml	100 ml
Luciferase Reaction Substrate II (50×)	100 µl	400 µl	2 ml
Cell Lysis Buffer (5×)	5 ml	20 ml	100 ml

Procedures

Self-prepared

Product Name	Catalog
PBS(1×)	TransGen, Cat. FG701-01
Nuclease-free Water	TransGen, Cat. GI101-01
Grinding balls or a mortar and pestle	

1. Reagent Preparation

Take out Luciferase Reaction Buffer and Luciferase Reaction Buffer II from -20°C. Bring reagents to room temperature and thaw them completely. (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). After aliquoting, protect from light and store at -20°C or -70°C. Avoid repeated freeze-thaw cycles.

(2) Luciferase Reaction Reagent II

Mix Luciferase Reaction Substrate II with Luciferase Reaction Buffer II at a ratio of 1:49. After aliquoting, protect from light and store at -20°C or -70°C. Avoid repeated freeze-thaw cycles.

(3) 1×Cell Lysis Buffer

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

2. Sample Processing

a. Animal cells

(1) Adherent cells: Remove the cell culture medium. Carefully rinse twice with 1×PBS, and add an appropriate amount of 1×Cell Lysis Buffer. Incubate at room temperature for 10 minutes for complete lysis. Scrape the cells into a 1.5 ml microcentrifuge tube.
(2) Suspension cells: Collect the cells and centrifuge at 300×g for 5 minutes. Remove the culture medium. Add an appropriate amount of 1× Cell Lysis Buffer, mix thoroughly by pipetting, and incubate at room temperature for 10 minutes for complete lysis.

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) Centrifuge at 12,000×g for 10 minutes at 2-8°C. Take the supernatant for use.
b. Plant leaves (using tobacco leaf discs as an example)
(1) Place 3-4 tobacco leaf discs (6-8 mm in diameter) into a 2 ml microcentrifuge tube. Add 3-4 pre-cooled grinding balls and an appropriate amount of liquid nitrogen. Grind the tissue using a tissue homogenizer.
Alternatively, place 3-4 tobacco leaf discs (6-8 mm in diameter) into a mortar. Add an appropriate amount of liquid nitrogen and grind thoroughly using a pestle.
(2) After grinding to a fine powder, add 300 µl of 1× Cell Lysis Buffer (use 100 µl of lysis buffer per leaf disc). Mix thoroughly by pipetting and and incubate at room temperature for 5 minutes for complete lysis.
(3) Centrifuge at 12,000 × g for 2 minutes at 2-8°C. Collect the supernatant for use.
c. Protoplasts
(1) Collect the protoplasts and perform a cell count. Centrifuge at 300 × g for 5 minutes and carefully remove the supernatant.
(2) Add 100 µl of 1× Cell Lysis Buffer per 10^5 protoplasts. Mix thoroughly by pipetting and incubate at room temperature for 10 minutes for complete lysis.
(3) Centrifuge at 12,000 × g for 2 minutes at 2-8°C. Collect the supernatant for use.

3. Fluorescence Detection

Add 100 µl of Luciferase Reaction Reagent equilibrated to room temperature into a 1.5 ml microcentrifuge tube or opaque 96-well plate. Carefully pipette 20 µl of 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 µl of Luciferase Reaction Reagent II equilibrated to room temperature into a 1.5 ml microcentrifuge tube or opaque 96-well plate. 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.
- Luciferase Reaction Reagent and Luciferase Reaction Reagent II should be equilibrated to room temperature before use.
- 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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Service telephone +86-10-57815020
Service email custserv@transgenbiotech.com