# *TransDetect*® Dual-Luc Pro Luciferase Reporter Assay Kit

**Please read the manual carefully before use.**

**Cat. No.** FR203

**Storage:** The unopened kit can be stored at -18°C or below for one year. The reconstituted Dual-Luc Pro Luciferase Reaction Reagent can be stored at -70°C or below for one year, or at -18°C or below for no more than one month, and avoid repeated freezing and thawing. Dual-Luc Pro Luciferase Reaction Reagent II should be freshly prepared before use.

## Description

The *TransDetect*® Dual-Luc Pro Luciferase Reporter Assay Kit is a highly sensitive, homogeneous dual-luciferase reporter detection kit with no irritating smell and exceptionally stable luminescent signals. This kit contains high-purity firefly luciferin and coelenterazine, enabling sequential detection of firefly luciferase and Renilla luciferase in the same sample: first, the firefly luciferase signal is measured using firefly luciferin as substrate, then the signal is quenched, followed by detection of Renilla luciferase luminescence using coelenterazine as substrate—allowing for dual-luciferase reporter assays. Compared to flash-type *TransDetect*® Double-Luciferase Reporter Assay Kit(FR201), this product is easier to use, enables add-read assay protocol without the need for removal of the medium, and provides more stable glow-type signal.

## Features

- Stable luminescent signal: Half-life is about 2 h (20-25°C), suitable for high-throughput detection.
- Accurate result: The Renilla luciferase gene is used as an internal control to correct for variations in cell number between wells and transfection efficiency.
- Easy to use: Without the need for removal of the medium, and the detection reagent can be added directly.
- No irritating smell.

## Kit Contents

| Component | FR203-01 (100 rxns) | FR203-02 (1000 rxns) |
|---|---|---|
| Dual-Luc Pro Luciferase Reaction Buffer | 10 ml | 100 ml |
| Dual-Luc Pro Luciferase Reaction Substrate (Lyophilized) | 1 vial | 1 vial |
| Dual-Luc Pro Luciferase Reaction Buffer II | 10 ml | 100 ml |
| Dual-Luc Pro Luciferase Reaction Substrate II (100×) | 100 µl | 1 ml |

## Self-prepared

Single/multi-channel pipettes, white or black opaque well plates, microplate reader with luminescence detection module

## Protocol

1. Reagent Preparation

Take out the Dual-Luc Pro Luciferase Reaction Buffer and Dual-Luc Pro Luciferase Reaction Buffer II from -18 °C or below and allow them to equilibrate to room temperature until completely thawed.

(1) Dual-Luc Pro Luciferase Reaction Reagent

Pour the entire contents of one bottle of Dual-Luc Pro Luciferase Reaction Buffer into the vial containing the Dual-Luc Pro Luciferase Reaction Substrate (lyophilized). Gently invert to mix until the substrate is fully dissolved. Aliquot according to experimental needs and store protected from light at -70 °C or below for long-term storage, or at -18 °C or below for short-term storage. Avoid repeated freeze-thaw cycles. Before each use, equilibrate the reagent to room temperature.

(2) Dual-Luc Pro Luciferase Reaction Reagent II

As needed for the experiment, mix Dual-Luc Pro Luciferase Reaction Substrate II (100×) with equilibrated Dual-Luc Pro

Luciferase Reaction Buffer II at a 1:100 ratio. Mix thoroughly and use immediately after preparation, keeping the mixture protected from light.

Example: To prepare 1 ml of Dual-Luc Pro Luciferase Reaction Reagent II, add 10 µl of Dual-Luc Pro Luciferase Reaction Substrate II (100×) to 1 ml of Dual-Luc Pro Luciferase Reaction Buffer II.

2. Luminescence detection

(1) Take out the cell culture plate from the incubator and let it stand for 10–20 minutes to equilibrate to room temperature.

(2) Firefly luciferase activity measurement: Add a volume of Dual-Luc Pro Luciferase Reaction Reagent equal to the volume of culture medium present in each well.

Example: For a 96-well plate, add 80 µl of reagent to 80 µl of medium per well. For a 384-well plate, add 20 µl of reagent to 20 µl of medium per well.

(3) Incubate the plate on a horizontal shaker at room temperature with gentle shaking for 10 minutes, then measure the firefly luminescence signal (F) using a microplate reader.

(4) Renilla luciferase activity measurement: Add a volume of Dual-Luc Pro Luciferase Reaction Reagent II equal to the volume of culture medium present in each well.

Example: For a 96-well plate, add 80 µl of reagent to 80 µl of medium per well. For a 384-well plate, add 20 µl of reagent to 20 µl of medium per well.

(5) Incubate the plate on a horizontal shaker at room temperature with gentle shaking for 10 minutes, then measure the Renilla luminescence signal (R) using a microplate reader.

3. Data Analysis

(1) According to experimental requirements, each cell culture plate should include background control, experimental, and control groups.

- Background control: Non-transfected cells.
- Experimental group: Transfected cells treated as required by the experiment.
- Control group: Transfected cells without treatment.

All groups are processed identically in the measurement steps.

(2) Calculate the ratio of the luminescence signal of the reporter gene to that of the internal reference gene for each well:

Experimental ratio = (experimental F− background F) / (experimental R − background R)

Control ratio = (control F − background F) / (control R − background R)

(3) Normalize the ratios of the experimental groups:

Relative ratio = Experimental ratio / Control ratio

**Notes**

- Dual-Luc Pro Luciferase Reaction Buffer II can be stored at room temperature for a long time, avoiding the need for long temperature equilibration before use.
- The reconstituted Dual-Luc Pro Luciferase Reaction Reagent can be stored at -18°C or below for one month. Try to avoid repeated freezing and thawing. For long-term storage, store it at -70°C or below.
- Dual-Luc Pro Luciferase Reaction Reagent II should be freshly prepared before use.
- Temperature has a great influence on the luciferase-luciferin reaction rate. Therefore, the cell culture system to be tested and the reagents used need to be completely equilibrated to the same room temperature to ensure the consistency of the test results before assaying. For high-throughput assay requirements and multi-well plate culture systems, the temperature equilibration time will be extended accordingly during operation. For stacked culture well plates, longer equilibration time is required. The consistency of assay between wells that are not fully equilibrated will be affected, which will makes the assay results unreliable.
- The mixing steps in the experiment should be gentle to avoid excessive bubbles that affect the luminescent values.

- Multi-well plates: It is recommended to use white or black opaque well plates for assaying. Different types of well plates have different effects on the assay results. The influence between wells of the black well plates is small, and the light intensity absorption of the luminescent signal is higher; the influence between wells of the white well plates is certain, and the light intensity of the luminescent signal will hardly be lost. The transparent well plates is conducive to the observation of cell status during cell culture, but the luminescence signal interference between each assay well is great. Appropriate well plates can be selected for cell culture and assay according to different experimental needs.
- To reduce operational error in experiment, it is recommended to use a multichannel pipette to add reagents when testing a large number of samples. Pay attention to whether the volume pipetted by each hole of the multichannel pipette is consistent.

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