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

TransDetect® Dual-Luc Pro Luciferase Reporter Assay Kit provides dual luciferase reporter gene assay system with high-sensitivity, no irritating smell, very stable and homogeneous fluorescence signal. The kit contains high-purity firefly luciferin and coelenterazine, which uses firefly luciferin as a substrate to detect the activity of the firefly luciferase, while quenching the fluorescence reaction, the activity of the Renilla luciferase reporter gene is detected by using coelenterazine as a substrate, thereby achieving detection of two reporter genes in a single sample. 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 Dual-Luc Pro Luciferase Reaction Buffer and Dual-Luc Pro Luciferase Reaction Buffer II from -18°C or below. Bring reagents to room temperature and thaw them completely.

(1) Dual-Luc Pro Luciferase Reaction Reagent

Add one bottle of Dual-Luc Pro Luciferase Reaction Buffer to the Dual-Luc Pro Luciferase Reaction Substrate (Lyophilized), and gently invert several times to fully dissolve the substrate. Aliquot it according to needs, store at -70°C or below for long term or -18°C or below for short term, protected from light and avoid repeated freezing and thawing. Equilibrate to room temperature before each use.

(2) Dual-Luc Pro Luciferase Reaction Reagent II

Mix Dual-Luc Pro Luciferase Reaction Substrate II (100×) with Dual-Luc Pro Luciferase Reaction Buffer II equilibrated to

room temperature at a ratio of 1:100, operate at room temperature and avoid light, and prepare this reagent freshly before use. For example: if preparing 1 ml Dual-Luc Pro Luciferase Reaction Reagent II, add 10 µl Dual-Luc Pro Luciferase Reaction Substrate II (100×) to 1 ml Dual-Luc Pro Luciferase Reaction Buffer II.

2. Assay Procedure

(1) Take out culture plate from the incubator and stay still for 10-15 minutes to equilibrate to room temperature.

(2) Measuring firefly luciferase activity: Add a volume of Dual-Luc Pro Luciferase Reaction Reagent equal to the culture medium volume to each well. For example, for 96-well plates, 80 µl of reagent is added to cells grown in 80 µl of medium. For 384-well plates, 20 µl of reagent is added to cells grown in 20 µl of medium.

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

(4) Measuring Renilla luciferase activity: Add a volume of Dual-Luc Pro Luciferase Reaction Reagent II equal to the culture medium volume to each well. For example, for 96-well plates, 80 µl of reagent is added to cells grown in 80 µl of medium. For 384-well plates, 20 µl of reagent is added to cells grown in 20 µl of medium.

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

3. Data Analysis

(1) Set background control, experimental group and control group in each culture plate according to the experimental requirements. The background control is untransfected cells, the experimental group is treated transfected cells, the control group is untreated transfected cells The detection steps for all groups are the same.

(2) Calculate the ratio of the luminescent signal of the reporter gene to be tested and the internal control reporter gene in each well.

The ratio of experimental group = (experimental group F - background control F)/(experimental group R - background control R).

The ratio of control group = (control group F - background control F)/(control group R - background control R).

(3) Use the ratio of the control group to normalize the ratio of each experimental group.

The expression fold of the reporter gene to be tested in the experimental group = the ratio of the experimental group / the ratio of the control group.

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.

For research use only, not for clinical diagnosis

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