

TransDetect® Double-Luciferase Reporter Assay Kit

Cat. No. FR201

Storage: at -20°C in dark for one year. Prepared luciferase assay reagents (Luciferase Reaction Reagent and 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

TransDetect® Double-Luciferase Reporter Assay Kit provides an efficient method to perform dual-reporter assays. In the assay, the activities of firefly (*Photinus pyralis*) and *Renilla* (sea pansy) luciferases are measured sequentially from a single sample. The firefly luciferase reporter is measured firstly by adding Luciferase Reaction Reagent to generate a luminescent signal. After quantifying the firefly luminescence, this reaction is quenched, and the *Renilla* luciferase reaction is initiated by adding Luciferase Reaction Reagent II to the same tube.

Kit Contents

Components	FR201-01 (50 rxns)	FR201-02 (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

1. Prepare Luciferase Reaction Reagent

Dissolve the lyophilized Luciferase Reaction Substrate (whole vial) by adding 5 ml of Luciferase Reaction Buffer to the vial. The prepared Luciferase Reaction Reagent can be aliquoted and stored at -20°C for one month or -70°C for one year.

2. Prepare Luciferase Reaction Reagent II

Mix Luciferase Reaction Substrate II with Luciferase Reaction Buffer II with the ratio of 1:50. The prepared Luciferase Reaction Reagent II can be aliquoted and stored at -70°C for one month. For best results, the Luciferase Reaction Reagent II should be prepared fresh before each use.

3. Prepare 1×Cell Lysis Buffer

Mix 5×Cell Lysis Buffer with ddH₂O with the ratio of 1:4. 1×Cell Lysis Buffer can be stored at 2-8°C for one month.

4. Prepare Cell Lysate

Wash cells twice with PBS and add appropriate volume of 1×Cell Lysis Buffer. Incubate at room temperature for 10 minutes and then scrape the cells. Centrifuge at 12,000×g for 10 minutes at 2-8°C. Transfer the supernatant to a new tube.

5. Assay

Mix 20 µl of lysate with 100 µl of Luciferase Reaction Reagent. Place the tube in luminometer to measure the luminescent signal of firefly luciferase. Then, add 100 µl of Luciferase Reaction Reagent II to the tube, place the tube in luminometer to measure the luminescent signal of *Renilla* luciferase. Record the data.

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