

TransDetect[®] Steady-Luc Firefly Luciferase Reporter Assay Kit

Please read the datasheet carefully prior to use.

Cat. No. FR102

Version No. Version 1.0

Storage: At -20°C in the dark for one year. The prepared Steady-Luc detection solution should be stored at -20°C for 30 days, avoiding repeated freezing and thawing. If not used for a long time, it is recommended to store it at -80°C.

Description

Steady-Luc Firefly Luciferase Reporter Assay Kit provides firefly luciferase reporter gene assay system with high-sensitivity, very stable and homogeneous fluorescence signal to measure quickly and efficiently intracellular luciferase expression without washing and collecting cells. The kit contains luciferin and optimized reaction reagents. This assay is based on luciferase system, the prepared Steady-Luc detection solution is directly added to the cell culture system to fully lyse the cells and release the luciferase, which can emit stable and detectable light signal.

The kit contains reaction buffer and substrate. Add an equal volume of the mixed Steady-Luc assay solution to the cell culture, and the signal can be detected in 3 minutes. The signal with high intensity and a half-life of more than 3 hours can be more suitable for high-throughput assays that have higher requirements for signal stability. It is easy to use, fast and efficient. And the signal is sensitive and stable.

Kit Contents

Component	FR102-01	FR102-02
Steady-Luc Firefly Luciferase Reaction Buffer	10 ml	100 ml
Steady-Luc Firefly Luciferase Reaction Substrate (Lyophilized)	1 vial	1 vial

Protocol

1. Reagent Preparation

- (1) Thaw reagent: Thaw Steady-Luc Firefly Luciferase Reaction Buffer at 2-8°C or room temperature. It can also be thawed in a water bath, and the water bath temperature should not exceed 25°C.
- (2) Steady-Luc assay solution preparation: Add the entire bottle of thawed Steady-Luc Firefly Luciferase Reaction Buffer to Steady-Luc Firefly Luciferase Reaction Substrate, and gently invert several times to fully dissolve the substrate. Note: Steady-Luc Firefly Luciferase Reaction Substrate (Lyophilized) may have a small amount of powder adhering to the bottle cap and bottle mouth. Before unscrewing the bottle cap, we recommend to pick up the bottle and tap the bottom of the bottle gently on the table to make the powder fall off as much as possible. Gently unscrew the cap, being careful not to lose the freeze-dried powder.
- (3) Equilibrate to room temperature: Equilibrate prepared Steady-Luc assay solution to room temperature before use.
- (4) Invert the solution 5 times to mix well before use.

2. Assay Procedure

- (1) Take out the cells from the incubator and place it at room temperature for at least 15 minutes to allow the culture plate to completely equilibrate to room temperature.
- (2) Add the same volume of Steady-Luc assay solution that has been equilibrated to room temperature as the cell culture. If the cells are cultured in a 96-well plate and the culture volume is 100 µl, add 100 µl of Steady-Luc assay solution to the cell culture.
- (3) Shake horizontally and mix for 3 minutes to fully lyse the cells before assay.

Notes

1. 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.
2. Temperature: 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.
3. When the solvent content of the drug to be tested is high, it may interfere with the luciferase reaction, thus affecting the chemiluminescence signal. The interference of the solvent can be eliminated by setting up cell culture medium containing solvent to culture cells that do not express luciferase as control wells.
4. Microplate reader: Steady-Luc Firefly Luciferase Reporter Assay Kit is compatible with microplate readers with luminescence detection modules. Due to the different settings and sensitivities of different microplate readers, the measured light signal intensity values will also be different, which may affect the detection. Select appropriate parameter settings.

Recommended Products

The main features and differences of the three one-step firefly luciferase reporter gene assay kits are shown in the table below. If the luminescence signal needs to be relatively intensity and the assay is completed within 40 minutes, it is recommended to use FR104-Bright-Luc. If the stability of the luminescence signal is particularly high and large batches or continuous operations are required, it is recommended to use FR102-Steady-Luc. If the intensity and stability of the luminescence signal are required, it is recommended to use FR103-One-Luc. Assay reagents can be flexibly selected according to experimental requirements and actual conditions.

Cat. No.	Product Name	Signal Intensity	Half-Life
FR102	Steady-Luc	++	>3 hours
FR103	One-Luc	++++	60 minutes
FR104	Bright-Luc	+++++	40 minutes

For research use only, not for clinical diagnosis.

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