TOOLStable Cell Viability Reagent

Cat. no.: TTC-DDAA01-10/100

Product Size: 10 mL / 100 mL

Storage: The TOOLStable Cell Viability Reagent should be maintained at −20 °C for long-term storage (TOOLStable Cell Viability Reagent is stable over five freeze–thaw cycles). This product can be stored at 2–8 °C for 60 days (>85% activity) and at room temperature for 7 days.

Introduction

The TOOLStable Cell Viability Reagent is a luciferin/luciferase-based system for detecting cell viability. The ready-to-use reagent in this kit contains luciferin and thermally stable luciferase. The intensity of luminescence is directly proportional to the amount of ATP present, and the amount of ATP is directly proportional to the number of viable cells within a certain range. The addition of the aforementioned reagent to lyse cells leads to ATP production, and the intensity of luminescence is proportional to the ATP content (number of live cells) in a certain range. The aforementioned reagent kit is suitable for measuring cell viability.

Features

This product is ready to use. Add a volume of TOOLStable Cell Viability Reagent equivalent to that of the culture medium to the cell culture plate. Measure the luminescence after 10 min. The half-life of luminescence is 4 hours. This product is suitable for high-throughput detection of cell proliferation and toxicity.

Additional materials required

A shaker, 96-well plate (black or white), and luminometer

Protocol

Reagent preparation

1. Thaw the TOOLStable Cell Viability Reagent at 2–8 °C, at room temperature, or in a 22 °C water bath (the temperature should not exceed 25 °C).
2. A 10-mL quantity (30 min for 100 mL) of the reagent requires 10 min to completely reach room temperature.
3. Mix well by gently inverting the bottle five times before use.

Detection

1. Maintain the cell culture plate at room temperature for 30 min.
2. Add a volume of a TOOLStable Cell Viability Reagent equivalent to that of the culture medium to the cell culture plate. For example, add 100 μL of reagent into a well containing 100 μL of culture medium (96-well plate).

Note: Equilibrate the TOOLStable Cell Viability Reagent to room temperature before mixing it with the cell culture medium

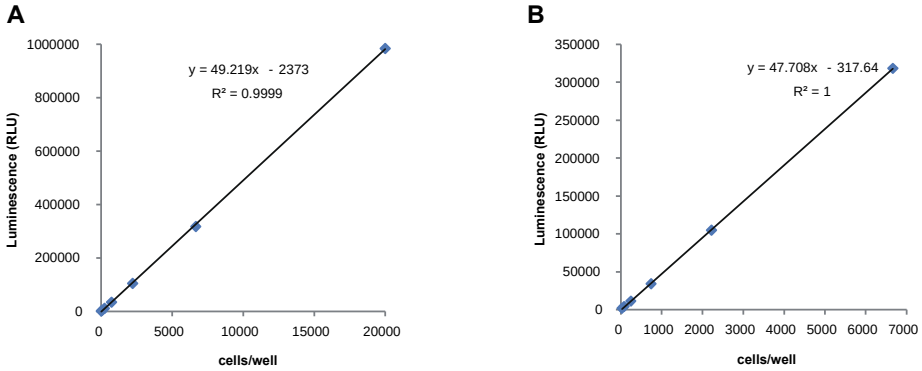
3. To lyse the cells, mix well by shaking the plate for 2–5 min. To emit stable luminescence, maintain the plate at room temperature for 10 min.
4. Measure the luminescence signal.

Important notes

1. Temperature directly affects the luciferase activity, which affects the luminescence intensity. This product and the cell culture must be maintained at room temperature before loading to maintain the consistency of the test results. Stacked 96-well plates require more time to equilibrate to room temperature than one 96-well plate does. Insufficient equilibrium may cause unevenness in the temperature of the stacked plates, which results in a gradient effect between the center and edge of the wells in the plates.
2. The chemical composition of various media are different. The intensity and rate of decay of the luminescence change marginally when using different types of media and serum. In addition, the solvent introduced by the compound used to treat the cells may also affect luminescence. Solvent interference can be eliminated by using a medium control well containing the solvent. When the final concentration of commonly used solvents, such as DMSO, methanol, and ethanol, is <2%, they have no significant effect on the luminescent signal.
3. We recommend the use of flat-bottom nontransparent 96-well plates. The intensity of the measured luminescence varies according to the type of plates used. Black 96-well plates exhibit low well–well interference but cause a large luminescence loss. White 96-well plates exhibit relatively high

well–well interference but do not cause a large luminescence loss. A specific type of 96-well plate should be selected according to experimental needs.

4. Complete mixing of the TOOLStable Cell Viability Reagent and cells results in enhanced luciferase activity and more accurate luciferase activity measurement. The aforementioned mixing is beneficial for cell lysis and ATP release. Suspension cell cultures exhibit a higher mixing efficiency than adherent cells do. Moreover, a 96-well plate exhibits a higher mixing efficiency than a 384-well plate because the well size and liquid depth of the plate affect the mixing efficiency. Users should optimize the mixing or shaking procedure according to the cell culture conditions and lab equipment.
5. Microbial contamination in the environment introduces exogenous ATP, which causes an increase in the background signal. We recommend wearing a mask and latex gloves during operation. Please keep the lab or detection area clean and open the cover carefully to avoid contamination.



Linear relation between cell number and luminescence

Cell suspensions of various densities are prepared by serially diluting Jurkat cells three times in RPMI 1640 medium containing 10% FBS. Add 100 μL of the cell suspension to a 96-well plate (Fig. A) or add 25 μL of the cell suspension to a 384-well plate (Fig. B). TOOLStable Cell Viability Reagent is added to the suspension in a 1:1 v/v ratio, and the luminescence is detected after standing the mixture for 10 min.

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