CytoTell™ UltraGreen

Catalog number: 22240, 22241
Unit size: 500 Tests, 2x500 Tests

Component	Storage	Amount (Cat No. 22240)	Amount (Cat No. 22241)
CytoTell™ UltraGreen	Freeze (< -15 °C), Minimize light exposure	500 Tests	1000 Tests

OVERVIEW

Flow cytometry combined with fluorescence staining is a powerful tool to analyze heterogeneous cell populations. Among all the existing fluorescent dyes CFSE is the preferred cell proliferation indicator that is widely used for live cell analysis. However, there are a few severe problems associated with the use of CFSE for monitoring cell proliferation. 1). CFSE is highly toxic to cells. CFSE indiscriminately reacts with all amino groups, thus changes many critical intracellular protein functions (such as cell membrane GPCRs); 2). CFSE has slow response and is inconvenient to use. The CFSE fluorescence intensity of the 2nd generation cells is decreased more than 10 fold from the 1st generation. You would have to wait for another generation to start the cell proliferation analysis. 3). Medium removal is required. You would have to remove medium for cell analysis with a flow cytometer since CFSE reacts with medium components. CytoTell™ Green has been developed to eliminate these CFSE limitations. Based on our customers' feedbacks on our CytoTell™ Green, CytoTell™ UltraGreen is our newest improvement. It has distinct advantages. 1). CytoTell™ UltraGreen is well retained in cells; 2). CytoTell™ UltraGreen exhibits much faster response and is more convenient to use than CFSE. The fluorescence intensity gap between 1st and 2nd generation is significantly minimized. As cells divide, CytoTell™ UltraGreen is distributed equally between daughter cells that can be measured as successive halving of the fluorescence intensity of the dye; 3). CytoTell™ UltraGreen is more sensitive than CFSE. Up to 9 generations may be visualized; 4). CytoTell™ UltraGreen is much more stable than CFSE. CytoTell™ UltraGreen stock solution can be stored at room temperature for a few days. CytoTell™ UltraGreen can also be used for long term tracking of labeled cells. Analysis using two-parameter plots may provide better resolution of each generation, especially between undivided cells and the first generation. CytoTell™ UltraGreen has a peak excitation of 519 nm and can be excited by the blue (488 nm) laser line, making it compatible with FITC filter set.

AT A GLANCE

Protocol Summary

1. Prepare cells with test compounds
2. Add 1X dye working solution
3. Incubate dyes with cells at room temperature or 37 ° C for 10 to 30 minutes
4. Remove the dye working solution
5. Analyse with flow cytometer with appropriate filter set

Important Bring all the kit components at room temperature before starting the experiment. *Note:* The CytoTell™ dyes are lyophilized powders. They should be stable for at least 6 months if store at -20 °C, protecting from light, and avoiding freeze/thaw cycles.

KEY PARAMETERS

Flow cytometer

Excitation	488 nm laser
Emission	530/30 nm filter
Instrument specification(s)	FITC channel

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

CytoTell™ dye stock solution (500X)
Add 500 µL DMSO into the dye powder vial, mix it well by vortexing to have a stock solution (500X). *Note: The stock solution should be used promptly; any remaining solution should be aliquoted and frozen at < - 20 ° C. Avoid repeated freeze-thaw cycles, and protect from light.*

PREPARATION OF WORKING SOLUTION

CytoTell™ dye working solution (1X)
Dilute the 500X DMSO stock solution at 1 to 500 in Hanks and 20 mM Hepes buffer (HHBS) or the buffer of your choice, pH 7 (such as 1 µL of 500X DMSO stock solution to 500 µL buffer) right before use. Mix them well by vortexing. *Note: The final concentration of the dye working solution should be empirically determined for different cell types and/or experimental conditions. It is recommended to test at the concentrations that are at least over ten fold range. Such as CytoTell™ Red might use much less amount in some cell types than the recommend concentrations.*

SAMPLE EXPERIMENTAL PROTOCOL

1. Treat cells with test compounds for a desired period of time.
2. Centrifuge the cells to get 1-5 × 10^5 cells per tube.
3. Resuspend cells in 500 µL of the CytoTell™ dye working solution. Optional: One can add the 500X DMSO stock solution into the cells directly without medium removing (such as, add 1 µL500X DMSO stock solution into 500 µL cells)
4. Incubate cells with a dye solution at room temperature or 37 °C for 10 to 30 minutes, protected from light.
5. Remove the dye working solution from the cells, wash the cells with HHBS or buffer of your choice. Resuspend cells in 500 µL of pre-warmed HHBS or medium to get 1-5 × 10^5 cells per tube.
6. Monitor the fluorescence change at respected Ex/Em (see Table 1) with a flow cytometer or a fluorescence microscope.

EXAMPLE DATA ANALYSIS AND FIGURES

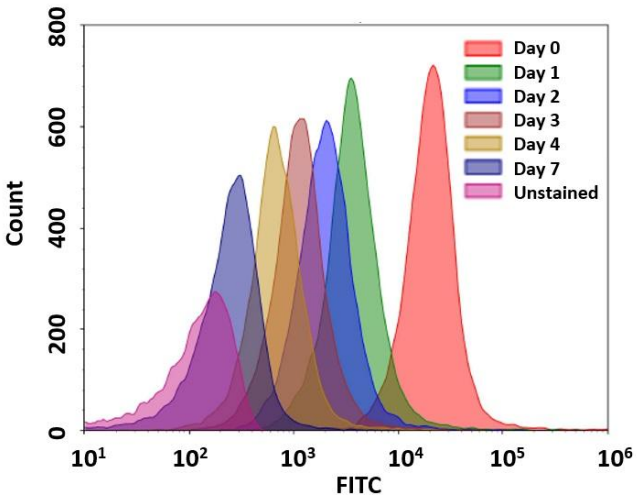

Figure 1. Cell tracking assay using CytoTell™ UltraGreen. Jurkat cells (~2x10^6 cells/mL) were stained with CytoTell™ UltraGreen on Day 0. Cells were passed serially at 1:1 ratio for 7 days. Fluorescence intensity was measured using ACEA NovoCyte flow cytometer in FITC channel. Successive generations were represented by different colors.

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