

CytoTell® Green

Catalog number: 22253, 22254
Unit size: 500 Tests, 2x500 Tests

Component	Storage	Amount (Cat No. 22253)	Amount (Cat No. 22254)
CytoTell® Green	Freeze (< -15 °C), Minimize light exposure	500 Tests	2x500 Tests

OVERVIEW

CytoTell® Green is a fluorescent cell proliferation indicator developed for monitoring cell division and long-term tracking of live cells by flow cytometry and fluorescence microscopy. As cells divide, the dye is distributed equally between daughter cells, resulting in progressive halving of fluorescence intensity that enables visualization of successive cell generations. CytoTell® Green exhibits peak excitation around 510 nm and can be efficiently excited with the 488 nm blue laser line, making it compatible with standard FITC filter sets, fluorescence microscopes, flow cytometers, and high-content imaging systems. CytoTell® Green was developed to address several limitations associated with CFSE-based proliferation assays. Compared to CFSE, CytoTell® Green exhibits reduced cytotoxicity, improved intracellular retention, and simplified staining workflows since it does not react significantly with common medium components. The dye can be used for long-term tracking of labeled cells and may be fixed and permeabilized for intracellular target analysis using standard formaldehyde-containing fixatives and saponin-based permeabilization buffers.

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 Note

Bring all the kit components to room temperature before starting the experiment.

Note: The CytoTell® dyes are lyophilized powders. They should be stable for at least 6 months if stored at -20 °C, protecting from light and avoiding freeze/thaw cycles.

KEY PARAMETERS

Flow cytometer

Emission	530/30 nm filter
Excitation	488 nm laser
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, and 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 tenfold range. Such as, CytoTell® Red might use much less amount in some cell types than the recommended concentrations.

SAMPLE EXPERIMENTAL PROTOCOL

1. Treat cells with test compounds for a desired period of time.
2. Centrifuge the cells to get $1-5 \times 10^5$ 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 µL 500X 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 $1-5 \times 10^5$ cells per tube.
6. Monitor the fluorescence change at respected Ex/Em (see Key Parameters) with a flow cytometer or a fluorescence microscope.

EXAMPLE DATA ANALYSIS AND FIGURES

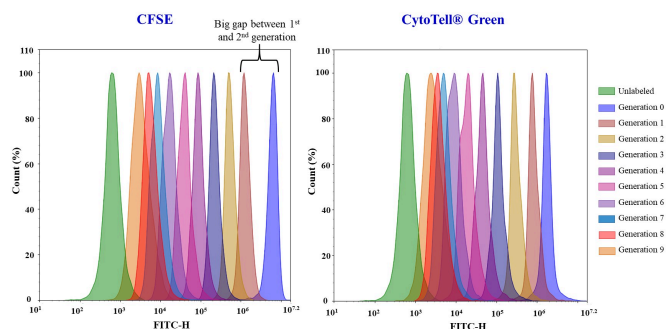


Figure 1. Cell proliferation analysis of Jurkat cells stained with CytoTell™ Green or CFSE. Cells were labeled on Day 0 and serially passaged at a 1:1 ratio for 9 days. The fluorescence intensity of each cell generation was monitored using an ACEA NovoCyte 3000 flow cytometer in the FITC channel. Successive cell generations are represented by different colors.

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