

## 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

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 is developed to eliminate these limitations. It has distinct advantages over CFSE. 1). CytoTell™ Green has very minimal cytotoxicity compared to CFSE. It is well retained in cells since the probe is designed to minimize the MDR interaction that generally pumps many cell stains out cells; 2). CytoTell™ Green exhibits much faster response and is more convenient to use than CFSE. There is no fluorescence intensity gap between 1st and 2nd generation of cells. As cells divide, CytoTell™ Green is distributed equally between daughter cells that can be measured as successive halving of the fluorescence intensity of the dye; 3). There is no need to remove medium since CytoTell™ Green does not react with medium components; 4). CytoTell™ Green is more sensitive than CFSE. Up to 9 generations may be visualized; 5). CytoTell™ Green is much more stable than CFSE. CytoTell™ Green stock solution can be stored at room temperature for a few days. CytoTell™ Green 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. Cells labeled with CytoTell™ Green may be fixed and permeabilized for analysis of intracellular targets using standard formaldehyde-containing fixatives and saponin-based permeabilization buffers. CytoTell™ Green has a peak excitation of 520 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 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)

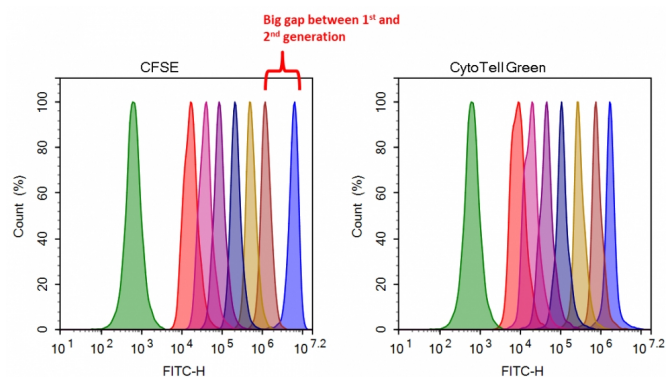
Dilute the 500X DMSO stock solution at 1 to 500 in Hanks and 20 mM Hepes buffer (HHBS) or the buffer of your choice, pH 7 (such as 1 µL of 500X DMSO stock solution to 500 µL buffer) right before use. Mix them well by vortexing.

**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 assay with CytoTell™ Green and CFSE.

Jurkat cells ( $\sim 2 \times 10^6$  cells/mL) were stained with CytoTell™ Green or CFSE on Day 0. The cells were passed serially at 1:1 ratio on the day specified. Fluorescence intensity was measured with ACEA NovoCyte 3000 flow cytometer in FITC channel on the day of passage. Successive generations were represented by different colors.

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