CytoTell™ Violet 500

Catalog number: 22248, 22249
Unit size: 500 Tests, 1000 Tests

Component	Storage	Amount (Cat No. 22248)	Amount (Cat No. 22249)
ThiolTrace™ Violet 500	Freeze (< -15 °C), Minimize light exposure	500 Tests	1000 Tests

OVERVIEW

Flow cytometry combined with fluorescence staining is a powerful tool for analyzing heterogeneous cell populations. CFSE is the preferred cell proliferation indicator widely used for live cell analysis among all the existing fluorescent dyes. However, it is impossible to use CFSE and its fluorescein analogs for GFP-transfected cells or for applications where a FITC-labeled antibody is used since CFSE and its fluorescein analogs have the excitation and emission spectra almost identical to GFP or FITC. CytoTell™ dyes are well excited at major laser lines such as 405 nm, 488 nm, or 633 nm with multicolor emissions. CytoTell™ dyes have minimal cytotoxicity and are used for multicolor applications with either GFP cell lines or FITC-labeled antibodies since they have either excitation or emission spectra distinct from fluorescein. CytoTell™ Violet 500 is a violet laser-excitable green fluorescent dye that stains cells evenly. As cells divide, the dye is distributed equally between daughter cells, which can be measured as a successive halving of the fluorescence intensity of the dye. Cells labeled with CytoTell™ Violet 500 may be fixed and permeabilized to analyze intracellular targets using standard formaldehyde-containing fixatives and saponin-based permeabilization buffers. CytoTell™ Violet 500 has a peak excitation of 405 nm and can be excited by the violet (405 nm) laser line. It has a peak emission of 500 nm and can be detected with a 500/20 bandpass filter (equivalent to BD Horizon® V500), making it compatible with applications that utilize GFP or FITC antibodies for multicolor cell analysis.

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 ^{o}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 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

Emission	525/50 nm filter
Excitation	405 nm laser
Instrument specification(s)	Pacific Orange 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 ^{o}C. Avoid repeated freeze-thaw cycles, and protect from light.

PREPARATION OF WORKING SOLUTION

CytoTellTM 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

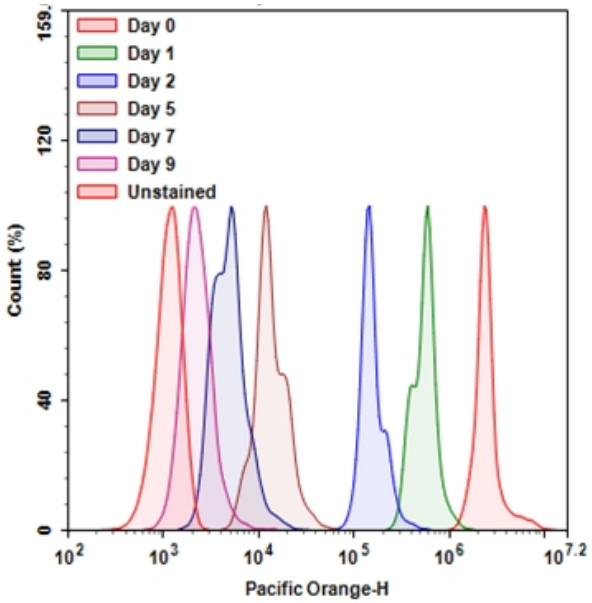

Figure 1. Cell proliferation assay with CytoTell™ Violet 500. Jurkat cells (~2×10^6 cells/mL) were stained with CytoTell™ Violet 500 on Day 0. The cells were passed serially at 1:1 ratio on the day specified. Fluorescence intensity was measured with ACEA NovoCyte 3000 flow cytometer with Pacific Orange channel. Successive generations were represented by different colors.

DISCLAIMER

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