CytoTell™ Orange

Catalog number: 22257, 22258
Unit size: 500 Tests, 2x500 Tests

Component	Storage	Amount	
		Cat No. 22257	Cat No. 22258
CytoTell™ Orange	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, it is impossible to use CFSE and its fluorescein analogs for GFP-transfected cells or for the 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 the multicolor applications with either GFP cell lines or FITC-labeled antibodies since they have either excitation or emission spectra distinct from fluorescein. CytoTell™ Red is a red fluorescent dye that stains cells evenly. As cells divide, the dye is distributed equally between daughter cells that can be measured as successive halving of the fluorescence intensity of the dye. Up to 8 generations may be visualized. CytoTell™ Orange 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™ Orange may be fixed and permeabilized for analysis of intracellular targets using standard formaldehyde-containing fixatives and saponin-based permeabilization buffers. CytoTell™ Orange has a peak excitation of 542 nm and can be excited by the blue (488 nm) and green (532 nm) laser lines. It has a peak emission of ~560 nm and can be detected with a 575/26 band pass filter (equivalent to RPE).

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.

Product Number	Indicator	Size	Ex/Em (nm)	Excitation Source
22240	CytoTell™ UltraGreen	500 tests	492/519	488 nm (Blue Laser)
22241	CytoTell™ UltraGreen	1000 tests	492/519	488 nm (Blue Laser)
22248	CytoTell™ Violet 500	500 tests	415/499	405 nm (Violet Laser)
22251	CytoTell™ Blue	500 tests	403/454	405 nm (Violet Laser)
22252	CytoTell™ Blue	1000 tests	403/454	405 nm (Violet Laser)
22253	CytoTell™ Green	500 tests	511/525	488 nm (Blue Laser)
22254	CytoTell™ Green	1000 tests	511/525	488 nm (Blue Laser)
22255	CytoTell™ Red 650	500 tests	628/643	633 nm (Red Laser)
22256	CytoTell™ Red 650	1000 tests	628/643	633 nm (Red Laser)
22257	CytoTell™ Orange	500 tests	542 /556	488 nm (Blue Laser) 531 nm (Green Laser)
22258	CytoTell™ Orange	1000 tests	542 /556	488 nm (Blue Laser) 531 nm (Green Laser)
22261	CytoTell™ Red 590	500 tests	560 /574	488 nm (Blue Laser) 531 nm (Green Laser)
22262	CytoTell™ Red 590	1000 tests	560 /574	488 nm (Blue Laser) 531 nm (Green Laser)

KEY PARAMETERS

Instrument:	Flow cytometer
Excitation:	488 nm or 532 nm laser
Emission:	575/26 nm filter
Instrument specification(s):	PE 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.

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EXAMPLE DATA ANALYSIS AND FIGURES

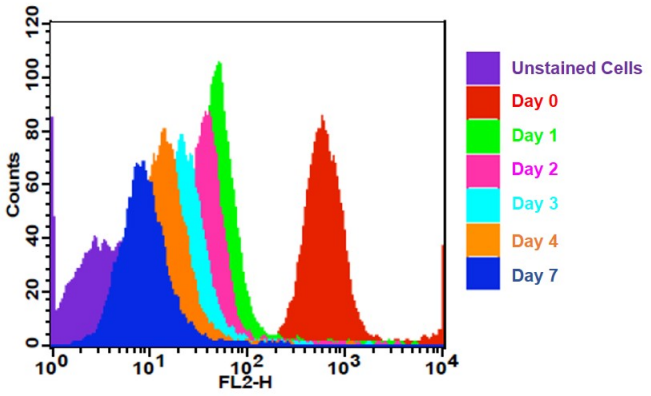

Figure 1. Cell tracking assay with CytoTell™ Orange. Jurkat cells (~2x10^6 cells/mL) were stained with CytoTell™ Orange on day 0. Cells were passed serially at 1:1 ratio for 7 days. Fluorescence intensity was measured using FACS Calibur flow cytometer in FL2 channel. Successive generations were represented by different colors.

DISCLAIMER