CytoTell® Blue

Catalog number: 22251, 22252
Unit size: 500 Tests, 2x500 Tests

Component	Storage	Amount (Cat No. 22251)	Amount (Cat No. 22252)
CytoTell® Blue	Freeze (< -15 °C), Minimize light exposure	500 Tests	2x500 Tests

OVERVIEW

CytoTell® Blue is a blue 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® Blue exhibits peak excitation around 405 nm and peak emission around 450 nm, making it compatible with violet laser excitation and detection using 450/20 bandpass filters commonly used for Pacific Blue® or BD Horizon® V450 channels. CytoTell® Blue was developed for multicolor applications where CFSE and other fluorescein-based proliferation dyes are not suitable due to spectral overlap with GFP or FITC-labeled antibodies. The dye exhibits minimal cytotoxicity and provides spectral separation from fluorescein-based probes for multiparameter cell analysis. CytoTell® Blue-labeled cells may be fixed and permeabilized for intracellular target analysis using standard formaldehyde-containing fixatives and saponin-based permeabilization buffers, making the dye suitable for downstream intracellular staining workflows and multicolor flow cytometry applications.

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.

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

Flow cytometer

Emission	450/40 nm filter
Excitation	405 nm laser
Instrument specification(s)	Pacific Blue 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

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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

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

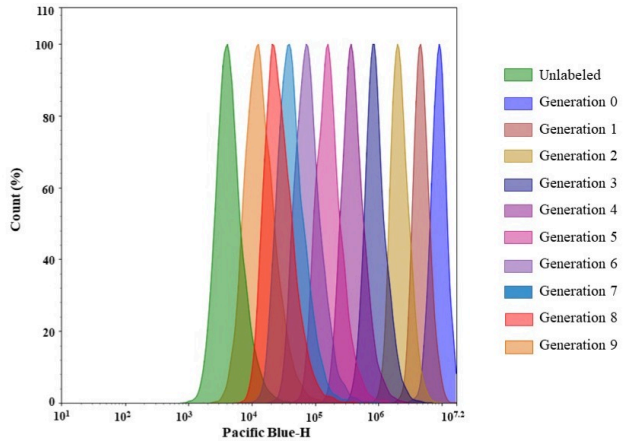

Figure 1. Cell proliferation analysis of Jurkat cells stained with CytoTell™ Blue. Cells were labeled on Day 0 and serially passaged at a 1:1 ratio for 9 days. The fluorescence intensity of each cell generation was monitored using an ACEA NovoCyte 3000 flow cytometer in the Pacific Blue channel.

DISCLAIMER

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