

## CytoTell™ Blue

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

Component	Storage	Amount	
		Cat No. 22251	Cat No. 22252
CytoTell™ Blue	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™ Blue is a blue 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. Cells labeled with CytoTell™ Blue may be fixed and permeabilized for analysis of intracellular targets using standard formaldehyde-containing fixatives and saponin-based permeabilization buffers. CytoTell™ Blue has a peak excitation of 405 nm and can be excited by the violet (405 nm) laser line. It has a peak emission of 450 nm and can be detected with a 450/20 band pass filter (equivalent to Pacific Blue®, or BD Horizon® V450), 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 °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:	405 nm laser
Emission:	450/40 nm filter
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.*

*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):*

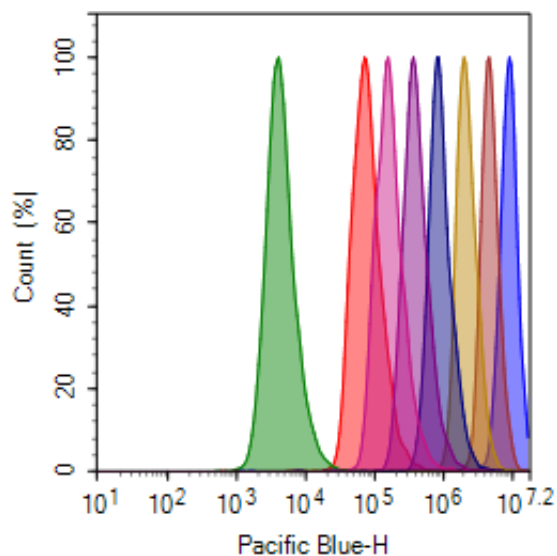
Dilute the 500X DMSO stock solution at 1 to 500 in Hanks and 20 mM Hepes buffer (HHBS) or the buffer of your choice, pH 7 (such as 1 µL of 500X DMSO stock solution to 500 µL buffer) right before use. Mix them well by vortexing.

**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  $1-5 \times 10^5$  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 Table 1) with a flow cytometer or a fluorescence microscope.

## EXAMPLE DATA ANALYSIS AND FIGURES



**Figure 1.** Cell proliferation assay with CytoTell™ Blue. Jurkat cells are stained with CytoTell™ Blue on Day0, and serially passed at 1:1 ratio for 8 days. Fluorescence intensity of each generation was measured with ACEA NovoCyte 3000 flow cytometer Pacific Blue channel.

## DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email [info@aatbio.com](mailto:info@aatbio.com) if you have any questions.