CytoTrace™ Ultra Green

Catalog number: 21800
Unit size: 1 mg

Component	Storage	Amount
CytoTrace™ Ultra Green	Freeze (<-15 °C), Minimize light exposure	1 mg

OVERVIEW

CMFDA (5-chloromethylfluorescein diacetate) has been used as a prominent cell tracker in the research community for a long time. We are introducing a new generation of fluorescent dye, named CytoTrace™ Ultra Green that is well suited for tracking the cell movement or location. The dye is well retained in cells, allowing tracking live cells for quite a few generations. Compared to CMFDA under the same conditions, CytoTrace™ Ultra Green is significantly brighter, more photostable and robust to use. Its fluorescence signal is also fixable. The signal generated by this dye is highly stable after fixation, making it ideal candidate to combine with other types of assays such as cytotoxicity etc for multicolor applications. The excitation and emission spectra of CytoTrace™ Ultra Green is identical to those of FITC, and are well separated from the common red fluorescent dyes such as Texas Red, Cy5, Cy7, iFluor™ 647 & 750, Alexa Fluor® 647 and 750. CytoTrace™ Ultra Green can be readily used for tracking live cells for various biological applications, and compatible with flow cytometry and fluorescence microscopy.

AT A GLANCE

Protocol summary

1. Prepare cells with test compounds
2. Add 0.5 to 5 µM CytoTrace™ Ultra Green working solution
3. Incubate dyes with cells at room temperature or 37 °C for 15 to 30 minutes
4. Remove the dye working solution
5. Analyze with a flow cytometer with Ex/Em = 490/520 nm-FITC filter set

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.

CytoTrace™ Ultra Green stock solution (2-10 mM):
Add appropriate amount of DMSO and mix well to make CytoTrace™ Ultra Green stock solution (2-10 mM).

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

CytoTrace™ Ultra Green working solution:
Prepare a 0.5 to 5 µM dye working solution right before use by diluting the DMSO stock solution from with Hanks and 20 mM Hepes buffer (HHBS) or the buffer of your choice, pH 7. Mix them well by vortexing.

Note In some cell types, lower concentration may be required to stain the cells. We recommend optimizing optimal concentration for each cell type before performing experiment.

SAMPLE EXPERIMENTAL PROTOCOL

1. Treat cells with test compounds for a desired period of time.
2. Centrifuge the cells to get 2-10 × 10^5 cells per tube.
3. Resuspend cells in 500 µL of the CytoTrace™ Ultra Green working solution.
4. Incubate cells with a dye solution at room temperature or 37 °C for 15 to 30 min, protected from light.
5. Remove the dye working solution from the cells; Optional: The cells can be fixed with 4% formaldehyde.
6. Wash the cells with HHBS or buffer of your choice once.
7. Resuspend cells in 500 µL of pre-warmed HHBS or medium to get 2-10 × 10^5 cells per tube.
8. Monitor the fluorescence change at Ex/Em = 490/520 nm with a flow cytometer or a fluorescence microscope with FITC filter set.

Product #	Indicator	Size	Molecular Weight	Ex/Em (nm)	Solvent
22014	CytoTrace™ Orange CMTMR	10x50 mg	554.04	541/565	DMSO
22015	CytoTrace™ Red CMPTX	10x50 mg	686.25	577/602	DMSO
22016	CytoTrace™ Red CFDA	1 mg	652.43	560/574	DMSO
22017	CytoTrace™ Green CMFDA	1 mg	464.86	494/521	DMSO
21800	CytoTrace™ Ultra Green	1 mg	822.72	494/521	DMSO
22020	FDA (Fluorescein diacetate)	1 g	416.83	494/521	DMSO

EXAMPLE DATA ANALYSIS AND FIGURES

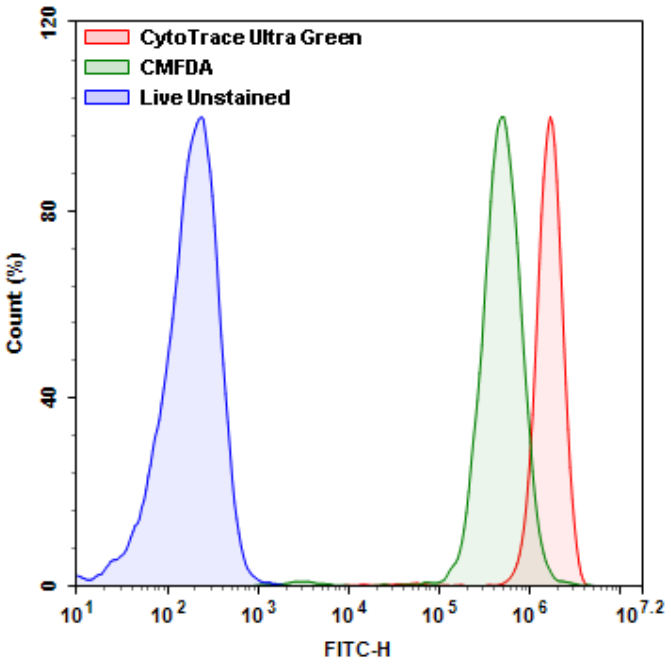

Figure 1. The comparison in the fluorescence intensity of CytoTrace™ Ultra Green

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with CMFDA in Jurkat cells. Jurkat cells were dye loaded with CytoTrace™ Ultra Green or CMFDA for 30 minutes in a 37 ^{o}C, 5% CO_2 incubator. The fluorescence intensity was measured using ACEA NovoCyte 3000 flow cytometer with FITC channel.

DISCLAIMER