Fluorescein Diacetate and CytoTrace™ Dyes

Introduction

FDA (Fluorescein Diacetate) is a non-fluorescent hydrophobic fluorescein derivative that can pass through cell membranes (including mammalian and bacteria cells) whereupon intracellular esterases hydrolyze the diacetate group producing the highly fluorescent product fluorescein. The fluorescein molecules accumulate in cells that possess intact membranes, serving as a marker of cell viability. Cells that do not possess an intact cell membrane or an active metabolism may not accumulate the fluorescent product and therefore do not exhibit green fluorescence. FDA may be used in combination with PI staining as the non-viable cells take up the PI and stain dead cells red whereas viable cells do not take up PI and should only stain green. This two-color separation of non-viable and viable cells may provide a more accurate quantitation of cell viability than single color analysis.

CytoTrace™ Green CMFDA is chemically same molecule to CellTracker™ Green CMFDA (Invitrogen C2925, CellTracker™ is the trademark of Invitrogen). CMFDA fluorescent probe retains the spectral properties of FITC, thus its signal can be typically monitored using fluorescence microscopy or flow cytometry. It freely passes through cell membranes and is converted to cell-impermeant products upon reactions with cellular components. The cell-impermeant reaction product is passed to daughter cells through several generations but is not transferred to adjacent cells in the population. Cells that are loaded with Cytotrace™ Green probe is typically fluorescent and viable for at least 24 hours, making this probe an excellent long-term cell tracer. The staining pattern can be fixed with formaldehyde or glutaraldehyde for signal amplification and other applications.

CytoTrace™ Orange CMTMR and CytoTrace™ Red CMTPX are chemically same to CellTracker™ Orange CMTMR and CellTracker™ CMTPX respectively. Both of them are highly fluorescent rhodamine dyes. CMTMR and CMTPX dyes freely pass through cell membranes into cells, where they are transformed into cell-impermeant reaction products. CMTMR and CMTPX dyes are retained in living cells through several generations. The dyes are transferred to daughter cells but not adjacent cells in a population. CMTMR and CMTPX dyes retain fluorescence for at least 72 hours, making them ideal cell tracking probes. CMTMR and CMTPX dyes are stable, nontoxic at working concentrations, well retained in cells, and brightly fluorescent at physiological pH. Additionally, the excitation and emission spectra of CMTMR and CMTPX dyes are well separated from GFP (green fluorescent protein) spectra allowing for multiplexing.

CytoTrace™ Red CFDA is a longer wavelength FDA analog. This unique probe is structurally similar to CytoTrace™ Green CMFDA, but with the longer wavelength spectral properties of Cy3/TRITC. Its signal can be easily monitored using fluorescence microscopy or flow cytometry. It freely passes through cell membranes and is converted to cell-impermeant products upon reactions with cellular components. The cell-impermeant reaction product is passed to daughter cells through several generations but is not transferred to adjacent cells in the population. Cells that are loaded with CytoTrace™ Red probe is typically fluorescent and viable for at least 24 hours, making this probe an excellent long-term cell tracer. The staining pattern can be fixed with formaldehyde or glutaraldehyde for signal amplification and other applications. Its spectra are well separated from those of GFP or FITC-labeled antibodies, making it an excellent choice for multiplexing with GFP cell lines or FITC-labeled antibodies.

Chemical and Physical Properties

Product #	Indicator	Size	Molecular Weight	Ex/Em (nm)	Solvent
22014	CytoTrace ™ Orange CMTMR	10×50 μg	554.04	541/565	DMSO
22015	CytoTrace™ Red CMTPX	10×50 μg	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
22020	FDA [Fluorescein diacetate]	1 g	416.83	494/521	DMSO

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Sample Assay Protocol

Brief Summary

Prepare cells with test compounds → Add 1 to 20 µM FDA working solution → Incubate dyes with cells at room temperature or 37 °C for 15 to 30 min → Remove the dye working solution → Analyze with a flow cytometer with Ex/Em = 490/520 nm (FL1 channel)

Note: The following is the recommended protocol. It only provides a guideline, should be modified according to the specific needs.

1. Prepare 2-10 mM DMSO stock solution

For #22014 add 45 uL DMSO into a 50 µg vial to make 2 mM stock solution (1 mg/ml is equivalent to 1.8 mM);
For #22015 add 36 uL DMSO into a 50 µg vial to make 10 mM stock solution (1 mg/ml is equivalent to 1.46 mM);
For #22016 add 153 uL ml DMSO to make 10 mM stock solution (1 mg/ml is equivalent to 1.53 mM);
For #22017 add 215 uL ml DMSO to make 10 mM stock solution (1 mg/ml is equivalent to 2.15 mM);
For #22020 dissolve 4.2 mg in 1 ml DMSO to make 10 mM stock solution (1 mg/ml is equivalent to ~ 2.4 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.

2. Prepare dye working solution

Prepare a 1 to 20 µM dye working solution right before use by diluting the DMSO stock solution from Step 1 with Hanks and 20 mM Hepes buffer (HHBS) or the buffer of your choice, pH 7. Mix them well by vortexing.

3. Analyze cells with a flow cytometer or a fluorescence microscope:

3.1 Treat cells with test compounds for a desired period of time.

3.2 Centrifuge the cells to get 2-10 × 10^5 cells per tube.

3.3 Resuspend cells in 500 µL of the dye working solution (from Step 2).

3.4 Incubate cells with a dye solution at room temperature or 37 °C for 15 to 30 min, protected from light.

3.5 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 2-10 × 10^5 cells per tube.

3.6 Monitor the fluorescence change at Ex/Em = 490/520 nm with a flow cytometer (FL1 channel) or a fluorescence microscope.

***Note: For bacterial cells staining**: Staining is most efficient when stock solution is diluted 1:800 in nutrient broth preconditioned by overnight growth of the test bacteria, but fresh nutrient broth or PBS may also be used. Bacterial suspensions should be diluted with PBS to 10^5 - 10^7 organisms per ml. Bacteria may be stained by applying one ml of solution to .45 µm filter (25mm) and vacuum filtering to remove solution, then adding 1ml of dye solution and incubating 5 - 10 minutes at room temperature.*

References

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