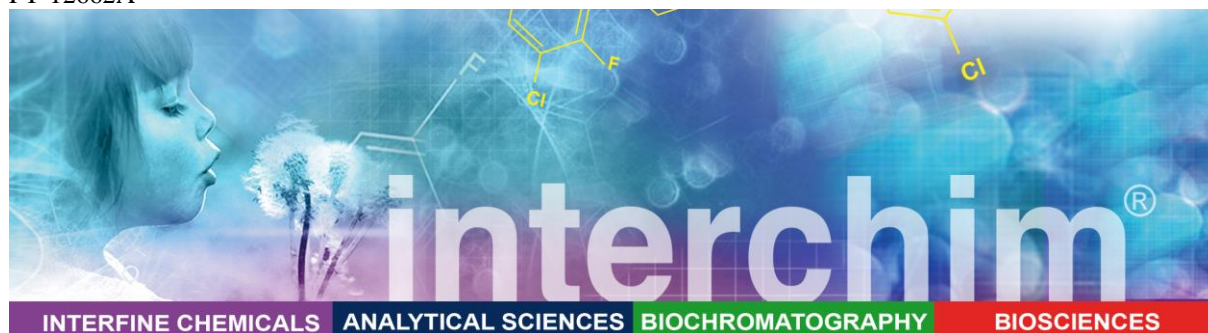


FT-12662A



INTERFINE CHEMICALS

ANALYTICAL SCIENCES

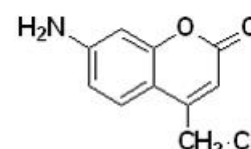
BIOCHROMATOGRAPHY

BIOSCIENCES

Cell Tracking reagents

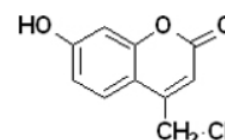
Products Information

Name :	CMAC Blue Cell Tracer 7-amino-4-chloromethylcoumarin, CAS: 147963-22-2 Cell tracker Blue, CellHunt Blue
Catalog Number :	FP-63323A, 5 mg ^(M)
Molecular Weight :	MW= 209.63
Soluble in:	DMSO, DMF, Methanol
Absorption / Emission :	$\lambda_{exc} \backslash \lambda_{em}$ (MeOH) = 353 / 466 nm
EC :	14 000 M ⁻¹ cm ⁻¹

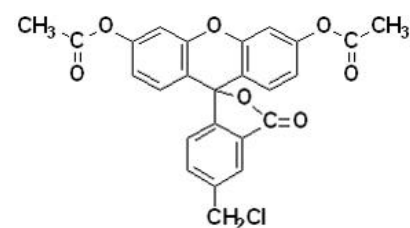


High Purity (>90%, typical 99%, by HPLC, by TLC).
Identity: NMR (d6-DMSO).

Name :	CMHC Blue Cell Tracer 4-Chloromethyl-7-hydroxycoumarin, CAS: 25392-41-0 Cell tracker Blue CMHC, CellHunt Blue
Catalog Number :	FP-46539A, 5 mg ^(M)
Molecular Weight :	MW= 210.61
Soluble in:	DMSO, DMF, Methanol
Absorption / Emission :	$\lambda_{exc} \backslash \lambda_{em}$ (MeOH) = 372 / 470 nm
EC :	16 000 M ⁻¹ cm ⁻¹



Name :	CMFDA Green Cell Tracer 5-chloromethylfluorescein diacetate; CAS: 136832-63-8 ; Spiro(isobenzofuran-1(3H),9'(9H)xanthen)-3-one, 3',6'-bis(acetyloxy)-5-(chloromethyl) Cell tracker Green, CellHunt Green
Catalog Number :	FP-38855A, 1 mg ^(K)

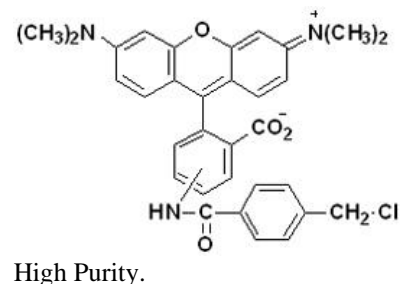


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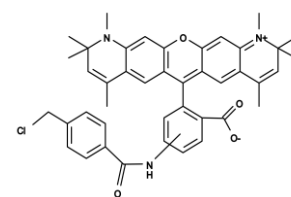
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Molecular Weight :	MW= 464.86	High Purity.
Soluble in:	DMSO, DMF, Acetonitrile and Chloroform	
Absorption / Emission :	$\lambda_{exc} \backslash \lambda_{em}$ (after hydrolysis) = 492 / 517 nm	
EC :	29 000 M ⁻¹ cm ⁻¹ (after hydrolysis)	

Name :	CMTMR Orange Cell Tracer	
	((4-chloromethyl)benzoyl amino)tetramethylrhodamine; CAS: 323192-14-9 Cell tracker Orange, CellHunt Orange	
Catalog Number :	FP-12662A, 1 mg	FP-12662C, 10x50 μ g ^(M)
Molecular Weight :	MW= 554.05	
Soluble in:	DMSO	
Absorption / Emission :	$\lambda_{exc} \backslash \lambda_{em}$ (MeOH) = 541 / 565 nm	
EC :	91 000 M ⁻¹ cm ⁻¹	



Name :	CMTPX Red Cell Tracer	
	Red CMPTX; Cell tracker Red	
Catalog Number :	FP-AK1965A, 10x50 μ g ^(M)	
Molecular Weight :	MW= 686.25	
Soluble in:	DMSO, DMF	
Absorption / Emission :	$\lambda_{exc} \backslash \lambda_{em}$ (after hydrolysis) = 577 / 602 nm	



Storage: -20°C >1 year or +4°C. ^(M) Protect from light and moisture

Technical information

● **CMTMR** is the most popular fluorescent dyes for reliable localization in long-term cell tracking studies. This fluorescent chloromethyl derivative freely enters in living cells, where it reacts mildly with thiol containing intracellular components and becomes highly fluorescent in cytoplasm at all physiological pH levels (low pKa). It localizes essentially in cytoplasm where it is converted to cell-impermeant probe. Fluorescent cells remain viable for at least 24 hours after loading, and it has been shown to be present in several cell generations up to two weeks.

It also can be fixed in situ with glutaraldehyde permitting long-term sample. CMTMR is widely used for long term cell tracing by cytometry, but also in 2 color imaging microscopy analysis thanks to its stable fluorescence. CMTMR can be used in combination with CMFDA.

● **CMAC** is a Fluorescent Thiol-Reactive cell permeant probe that easily pass through cell membranes. It can be used in co-incorporation with fluo-3 calcium indicator.

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● **CMFDA** is also a Thiol-Reactive, Cell permeant fluorescent probe that freely pass through cell membranes and it is converted to the highly fluorescent probe (fluorescein) once the the acetate groups are cleaved by intracellular esterases. It's signal is typically monitored using fluorescence microscopy. Cells that are loaded with this probe are 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.

● **CMTPX** freely passes through cell membranes into cells, where it is transformed into cell-impermeant reaction products. Red CMTPX dye is retained in living cells through several generations. The dye is transferred to daughter cells but not adjacent cells in a population. It displayd fluorescence for at least 72 hours, and the dye exhibits ideal tracking properties. The excitation and emission spectra of Red CMTPX dye are well separated from GFP (green fluorescent protein) spectra allowing for multiplexing.

Handling and Storage

CMAC, CMTMR, CMFDA and CMTPX are dissolved in anhydrous DMSO at 5-10 mM. Aliquote stock solution and stored at -20°C .

Directions for use

This protocol only provides a guideline, and should be modified according to your specific needs.

1. Prepare 2-10 mM DMSO stock solution

For CMTMR #FP-12662A add 45 μL DMSO into a 50 μg vial to make 2 mM stock solution (1 mg/ml is equivalent to 1.8 mM);

For CMTPX #FP-AK195A add 36 μL DMSO into a 50 μg vial to make 10 mM stock solution (1 mg/ml is equivalent to 1.46 mM);

For CFDA #FP-52493A add 153 μL ml DMSO to make 10 mM stock solution (1 mg/ml is equivalent to 1.53 mM);

For CMFDA #FP-38855A add 215 μL ml DMSO to make 10 mM stock solution (1 mg/ml is equivalent to 2.15 mM);

For # 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 $< -20^{\circ}\text{C}$. 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 to 7. Analyze cells with a flow cytometer or a fluorescence microscope:

3. Treat cells with test compounds for a desired period of time.
4. Centrifuge the cells to get $2-10 \times 10^5$ cells per tube.
5. Resuspend cells in 500 μL of the dye working solution (from Step 2).
6. Incubate cells with a dye solution at room temperature or 37°C for 15 to 30 min, protected from light.

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7. 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 x 10⁵ cells per tube.
8. 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 10⁵ - 10⁷ 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.

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Related products

Cell Biology assays > Cell tracing [\[BE030a\]](#)

- 5-CMF, FP-BT5160
- CFDA-SE, FP-52493A

Buffers

- HEPES, 061940

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