# Products Information

<table>
<thead>
<tr>
<th>Name:</th>
<th>CMAC Blue Cell Tracer</th>
<th><strong>CMHC Blue Cell Tracer</strong></th>
<th><strong>CMFDA Green Cell Tracer</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Catalog Number:</strong></td>
<td>FP-63323A, 5 mg (M)</td>
<td>FP-46539A, 5 mg (M)</td>
<td>FP-38855A, 1 mg (K)</td>
</tr>
<tr>
<td><strong>Molecular Weight:</strong></td>
<td>MW = 209.63</td>
<td>MW = 210.61</td>
<td>MW = 464.86</td>
</tr>
<tr>
<td><strong>Soluble in:</strong></td>
<td>DMSO, DFM, Methanol</td>
<td>DMSO, DFM, Methanol</td>
<td>DMSO, DMF, Acetonitrile and Chloroform</td>
</tr>
<tr>
<td><strong>Absorption / Emission:</strong></td>
<td>λ&lt;sub&gt;exc&lt;/sub&gt;/λ&lt;sub&gt;em&lt;/sub&gt; (MeOH) = 353 / 466 nm</td>
<td>λ&lt;sub&gt;exc&lt;/sub&gt;/λ&lt;sub&gt;em&lt;/sub&gt; (MeOH) = 372 / 470 nm</td>
<td>λ&lt;sub&gt;exc&lt;/sub&gt;/λ&lt;sub&gt;em&lt;/sub&gt; (after hydrolysis) = 492 / 517 nm</td>
</tr>
<tr>
<td><strong>EC:</strong></td>
<td>14 000 M⁻¹ cm⁻¹</td>
<td>16 000 M⁻¹ cm⁻¹</td>
<td>29 000 M⁻¹ cm⁻¹ (after hydrolysis)</td>
</tr>
</tbody>
</table>

**CMAC Blue Cell Tracer**

- 7-amino-4-chloromethylcoumarin, CAS: 147963-22-2
- Cell tracker Blue, CellHunt Blue

**CMHC Blue Cell Tracer**

- 4-Chloromethyl-7-hydroxycoumarin, CAS: 25392-41-0
- Cell tracker Blue CMHC, CellHunt Blue

**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
**CMTMR Orange Cell Tracer**

Name: (4-chloromethyl)benzoyl) amino)tetramethylrhodamine; CAS: 323192-14-9
Cel1tracker Orange, CellHunt Orange

**Catalog Number:** FP-12662A, 1 mg  
**Molecular Weight:** MW = 554.05
**Soluble in:** DMSO
**Absorption / Emission:** \(\lambda_{\text{exc}}\lambda_{\text{em}}\) (MeOH) = 541 / 565 nm
**EC:** 91 000 M\(^{-1}\) cm\(^{1}\)

**CMTPX Red Cell Tracer**

Name: Red CMPTX; Celltracker Red

**Catalog Number:** FP-AK1965A, 10x50µg
**Molecular Weight:** MW = 686.25
**Soluble in:** DMSO, DMF
**Absorption / Emission:** \(\lambda_{\text{exc}}\lambda_{\text{em}}\) (after hydrolysis) = 577 / 602 nm

**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.

- **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. Its signal is typically monitored using fluorescence microscopy.

- **CMTPX** freely passes through cell membranes into cells, where it is transformed into cell-impermeant reaction products. CytoTrace™ 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 displayed 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
FT-12662A
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°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 x10^5 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.
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 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 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.

References
- Brandt W., et al., A molecular mechanism for the cleavage of a disulfide bond as the primary function of agonist binding to G-protein-coupled receptors based on theoretical calculations supported by experiments », Eur. J. Biochem. 261, 89 (1999) Article
- Heredia A. et al., Rapamycin Reduces CCR5 Density Levels on CD4 T Cells, and This Effect Results in Potentiation of Enfuvirtide (T-20) against R5 Strains of Human Immunodeficiency Virus Type 1 In Vitro, Antimicrobial Agents and Chemotherapy, p. 2489-2496, Vol. 51, No. 7 (2007) Article
FT-12662A

**Related products**

Cell Biology assays > Cell tracing
- 5-CMF, FP-BT5160
- CFDA-SE, FP-52493A

Buffers
- HEPES, 061940

**Ordering information**

Catalog size quantities and prices may be found at [http://www.fluoprobes.com](http://www.fluoprobes.com)

Please inquire for higher quantities (availability, shipment conditions).

For any information, please ask : FluoProbes® / Interchim; Hotline: +33(0)4 70 03 73 06

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