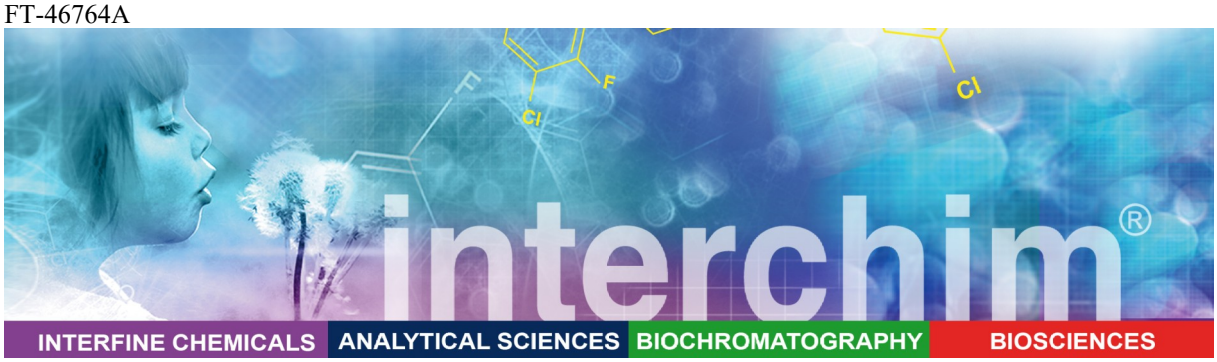
FluoProbes®

FT-46764A

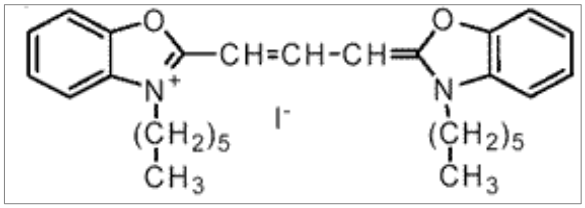


DiO dyes

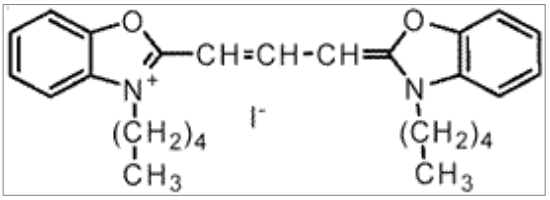
Lipophilic cationic dyes to stain internal membranes (mitochondria, endoplasmic reticulum) of live cells

Products Description

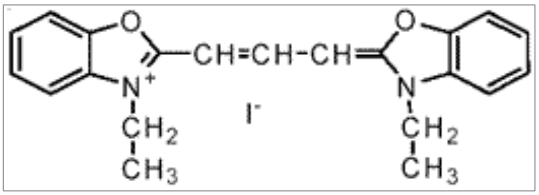
Name :	**$DiOC_6(3)$**
	3,3'-Dihexyloxacarbocyanine Iodide
Catalog Number :	FP-46764A, 100 mg Custom pack. & Bulk on inquire
Solubility:	in DMSO or DMF
Structure :	$C_{29}H_{37}IN_2O_2$; CAS:[53213-82-4]
Molecular Weight :	MW= 572.53
Absorption / Emission :	$\lambda_{exc}\backslash\lambda_{em}$ (in MeOH) = 484 / 501 nm
EC (M^{-1} cm^{-1}) **:**	150 000 $cm^{-1}M^{-1}$ (in MeOH)



Name :	**$DiOC_5(3)$** 3,3'-Dipentyloxacarbocyanine, iodide)
Catalog Number :	FP-468101, 25 mg FP-46810A, 100 mg
Structure :	$C_{27}H_{33}IN_2O_2$
Molecular Weight :	MW= 544.47
Solubility:	in DMSO or DMF
Absorption / Emission :	$\lambda_{exc}\backslash\lambda_{em}$ (in MeOH) = 482 nm/ 497 nm
EC (M^{-1} cm^{-1}) **:**	165 000 $cm^{-1}M^{-1}$ (in MeOH)
Quantum Yield :	0.04 (478nm)



Name :	**$DiOC_2(3)$** 3,3'-Diethyloxacarbocyanine iodide
Catalog Number :	FP-M20481, 100 mg
Structure :	$C_{21}H_{21}IN_2O_2$
Molecular Weight :	MW= 460.32
Solubility:	in DMSO or DMF
Absorption / Emission :	$\lambda_{exc}\backslash\lambda_{em}$ (in MeOH) = 482 nm/ 497 nm
EC (M^{-1} cm^{-1}) **:**	165 000 $cm^{-1}M^{-1}$ (in MeOH)
Quantum Yield :	0.04 (478nm)



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FT-46764A
Storage: +4°C (L) in a closed container, protected from moisture – or -20°C for long term

Introduction

$DiOC_2(3)$ $DiOC_5(3)$ and $DiOC_6(3)$ are carbocyanine derivatives with short alkyl tails (<7 carbon atoms). They accumulate on hyperpolarized membranes, by translocation into the lipid bilayer and aggregation that decreases their fluorescence. They were widely used for cell membrane potential measurements, including by flow cytometry and microscopy.

■ **$DiOC_6(3)$** is a green fluorescent membrane dye that is used to stain the ER (Golgi) in both live and fixed cells at high concentration, vesicle membranes and mitochondria. $DiOC_6$ can be used to label living cells, however they are quickly damaged due to photodynamic toxicity, so cells stained with this dye can only be exposed to light for short periods of time. The dye has been used to study structural interactions and dynamics of the ER in neurons and yeast. At lower concentration, the dye is more selective for the mitochondria (*)**.** It was also found that the nuclear envelope in yeast were specifically stained if the dye concentration was increased or in certain respiratory-deficient yeast strains, furthermore with sufficient sensitivity to reveal alterations in the nuclear envelope known as karmellae ([Koning 1993]). Finally cilia/flagella have been stained successfully r.

■ **$DiOC_5(3)$** is widely used carbocyanine dyes used for membrane potential measurements, in a similar way to $DiOC_6(3)$.

■ **$DiOC_2(3)$** is used to measure potential in bacteria ([Novo, 2000]), but also has been studied in some living cells as rat embryo fibroblasts, gerbil fibroma, monkey kidney cells , Chinese hamster lung fibroblasts, and mouse 3T3 fibroblasts ([Johnson, 1981]).

Note: A key difference between "cytoplasmic" and "mitochondrial" MP measurement with cationic dyes, including cyanines, is not only the dye concentration, but the wash step r.

Directions for use

Guidelines for use - in flow cytometry for cells staining with $DiOC_6(3)$

Optimization of the procedure may be necessary for each specific dye (i.e. other DiOC) and cell type.

1. Prepare a cells suspension at a density of ~ 1 x 10^6/ml in a serum - free culture medium or phosphate buffered saline.
2. Prepare a stock solution of $DiOC_2(3)$ or $DiOC_6(3)$ at 1 mM in DMSO, then a working solution at 10µM.
3. (optional) - control experiment using **CCCP** (carbonyl cyanide 3-chlorophenylhydrazone) that is a protonophore:
   Prepare a stock solution of CCCP at 50 mM.
   Make a control by mixing 1µl of 50mM CCCP and 5µl of 10µM cyanine dye with 1 ml of cells.
   Incubate 5 min at +37°C.
4. Incubate 1 ml of cells with 5µl of 10µM cyanine dye at 37°C for 15-30 min.
   Note: Temperature (RT, +37°C) and incubation time (a few minutes to 20 min) may vary depending on the cell types. In some protocol, in particular in culture cells, 5% CO_2 is recommended.
5. Separate the stained cells from the staining solution by centrifugation at +37 °C at 1 500 rpm for a few minutes.
6. Remove the supernatant and resuspend the cells in fresh medium, PBS.
7. Analyze with a flow cytometer.

Other protocols, particularly in fluorescent microscopy, may be found in the literature.

Guidelines for use - in microscopy for cells staining with $DiOC_6(3)$

$DiOC_6(3)$ can be used at 25µM as a starting final concentration. Refer to the literature ([Koning 1993, Lee 1995]). Useful concentration can vary depending on cell type, and organelle.

References

* Review
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* Fluorescence microscopy $DiOC_6(3)$
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-**Novo D.J**, *et al.*, « Multiparameter Flow Cytometric Analysis of Antibiotic Effects on Membrane Potential, Membrane Permeability, and Bacterial Counts of Staphylococcus aureus and Micrococcus luteus », *Antimicrobial Agents and Chemotherapy*, 44, 827(2000)

* $DiOC_5(3)$
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Safety information

Potentially harmful - First Aid measures :
Avoid prolonged or repeated exposure. Avoid getting in eyes, on skin, or on clothing. Wash thoroughly after handling.
If eye or skin contact occurs, wash affected areas with plenty of water for 15 minutes and seek medical advice. In case of inhaling or swallowing, move individual to fresh air and seek medical advice immediately.

Associated products

- FluoCD™ wash solution, FP-BC0991 – useful to clean tubular of cytometers for lowest residual background
- **FluoCD™** This technology is based on a proprietary cyclodextrin :
  Soluble in water! - no need DMSO. Just add water!
  Fluorescence **intensity increases** by 2-3 fold.
  Prevents dye aggregation *in situ*
  No cytotoxicity
- DiOC6(3) FluoCD™ stain for FCM, # FP-BC1001, 2ml 100X
- DiOC6(3) FluoCD™ stain for Microscopy # FP-BC1011, 1ml 100X

Just add 1ml of water to the stock solution of FluoCD™ $DiOC_6(3)$, it readily dissolves!
No need DMSO or other organic solvent that can be deleterious to cells or downstream applications, no additional dissolution steps is required (heating, sonication). Then using FluoCD™ $DiOC_6$ at the usual concentration of classic $DiOC_6$, you will get brighter signal! In microscopy you won't see unspecific artifacts dye to dye aggregation, i.e. it increases selectivity in staining mitochondria.

- CCCP, 091640 (carbonyl cyanide 3-chlorophenylhydrazone)

Related products

- Other lipophilic carbocyanine fluorescent dyes: FP Membrane Markers, including DiO (green fluorescence, such as DiOC1(3)), DiI (orange fluorescence, such as DiIC18(3)), DiD (red fluorescence, such as DiIC18(5)), DiR (deep red fluorescent, such as DiIC18(5)), and DiA

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- FluoCD™ technology is also available for other dyes: Green SynapTracer1-4 FP-BD1150 (FM-1-43), FP Membrane Marker4-64 FP-BC7250, DiIC18 ... Other dyes on inquire.

Ordering information

Catalog size quantities and prices may be found at 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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