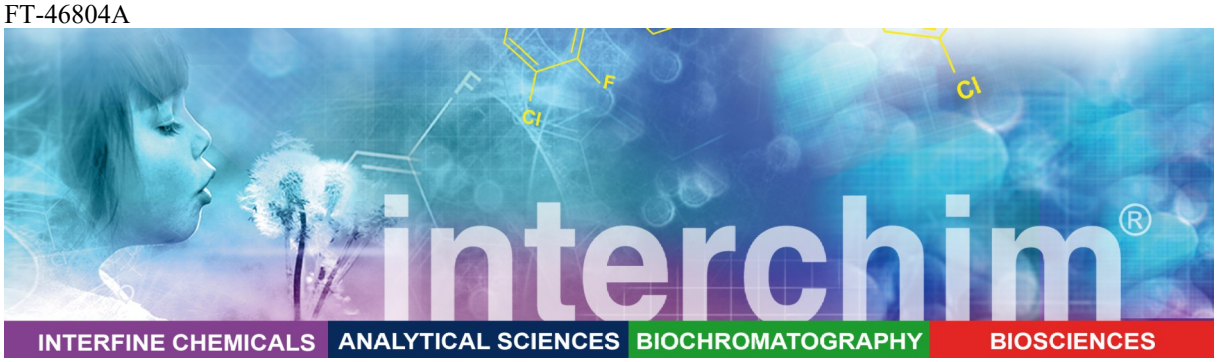
FluoProbes®

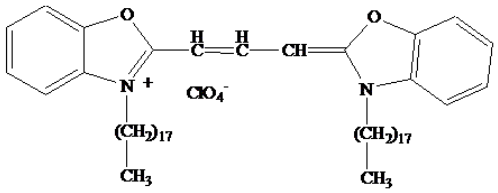
FT-46804A



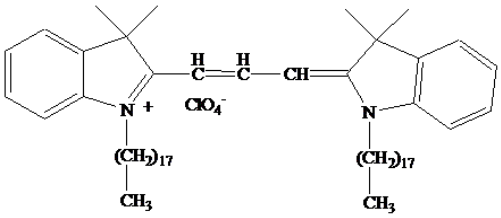
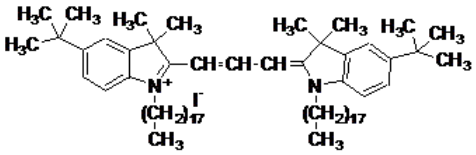
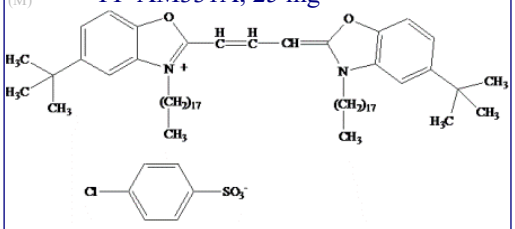
DiI, DiD, DiO, DiR, DiA, DiB

Lipophilic carbocyanine fluorescent dyes for membrane labeling

Product Information

cat.number	MW (g·mol^{-1}) CAS #	λ_{exc}\λ_{em} max. (nm)	Mol. abs. ($M^{-1}cm^{-1}$)	Soluble in
$DiOC_{18}(3)$ [DiO] (L) FP-46805A, 50 mg 	881.73 34215-57-1	484 / 501	154 000	DMF DMSO
$DiOC_6(3)$ (L) FP-46764A, 100 mg	572.53 53213-82-4	484 / 501	154 000	DMSO
$DiOC_{14}(3)$ (L) FP-AM329A, 50 mg	795.19	490 / 515		MetOh / EtOH DMSO
SP-$DiOC_{18}(3)$ (L) FP-40265A, 10 mg	1115.55	498 / 514	175 000	MetOh / EtOH DMSO
5,5'-Ph2-$DiOC_{18}(3)$ (L) FP-M1610A, 10 mg	969.91 217199-21-8	496 / 512		MetOh, DMF DMSO
$DiIC_1(3)$ (L) FP-46853A, 100 mg	484.42 25470-94-4	541 / 540		DMSO $CHCl_3$, DMF
$DiIC_1(5)$ (L) FP-20920A, 100 mg	432.25 36536-22-8	638/ 658		DMSO, DMF EtOH
$DiIC_1(7)$ (L) FP-C86280, 100 mg	509.04 16595-48-5	740 / 766		DMSO EtOH, $CHCl_3$
$DiIC_5(3)$ (L) FP-BT5040, 100 mg	596.63 53290-46-3	552 / 576		DMSO EtOH, DMF
$DiIC_{12}(3)$ (L) FP-46736A, 100 mg	765.56 75664-01-6	550 / 566	144 000	DMSO, DMF EtOH, $CHCl_3$
$DiIC_{16}(3)$ (L) FP-46746A, 100 mg	877.77 78566-75-3	548 / 566	148 000	DMSO EtOH

FT-46804A

cat.number	MW (g·mol^{-1}) CAS #	λ_{exc}\\λ_{em} max. (nm)	Mol. abs. ($M^{-1}cm^{-1}$)	Soluble in
DiIC$_{18}$(3) [DiI] (*) (L) FP-46804A, 50 mg 	933.88 41085-99-8	551 / 566	148 000	DMSO EtOH, CH_3CN
DiIC$_{18}$(3) [DiI], crystalline (L) FP-162451, 25 mg	933.88 41085-99-8	551 / 566	148 000	DMSO, DMF EtOH, $CHCl_3$
Dilinoleyl DiI Solid (L) FP-12792A, 5 mg	1017.97	549 / 564	134 600	DMSO
Neuro-DiI (L) FP-AM330A 25 mg 	1075.58	550 / 565	145 000 (MetOH)	DMSO / DMF MetOH / EtOH
Δ^9-DiI (L) FP-M1280A, 25 mg	925.49	550 / 565		DMSO, CH_3CN EtOH, DMF
6,6'-Ph2-DiIC$_{18}$(3) (L) FP-M1613A, 5 mg	1022.06 217199-28-5	557 / 572		DMSO, $CHCl_3$ EtOH, DMF
DiIC$_{18}$(5) [DiD] 4-chlorobenzenesulfonate salt (M) FP-22574A, 50 mg	1052.1	644 / 663	193 000	DMSO EtOH, DMF
DiIC$_{18}$(5) oil [DiD] perchlorate FP-929099, 10 mg FP-92909A, 25 mg	959.9 127274-91-3	644 / 665	270 000	$CHCl_3$, DMSO MeOH, acetone
DiIC$_{18}$(5) oil [DiD] iodide FP-DY3330, 25 mg	987.36 127274-91-3	644 / 665	270 000	DMSO
DiIC$_{18}$(7) [DiR] (M) FP-69084A, 25 mg	1013.43 100068-60-8	748 / 780 (MetOH)	270 000	DMSO EtOH
Neuro-DiO (**) (M) FP-AM331A, 25 mg 	1086.11	484 / 501	270 000	DMSO /EtOH Hexane, oil
Neuro-DiO (**) (M) FP-BA641A, 0,2 ml	1086.11	484 / 501	270 000	DMSO /EtOH Hexane, oil
DiA (L) FP-66096A, 25 mg see **FT-46720A (Styryl dyes)**	787.06	491 / 613	52 000 (MetOH)	DMSO and EtOH
DiB (L) FP-YS2860, 10 mg	1074	353 / 442		DMSO, DMF or EtOH

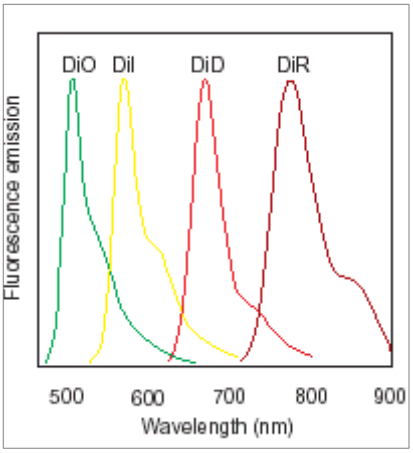
(*) DiIC$_{18}$(3) solution is also available for microinjections: FP-AM328A (0.5 ml)
(**)Neuro-DiO also is available in solution for microinjections: FP-BA641A (0.2 ml)

FT-46804A

Storage: $DiOC_{18}(3)$, $DiOC_{14}(3)$, DiI, Neuro-DiI, Dilinoleyl DiI solid, DiA, DiB can be stored at +4°C (L)
DiD, DiR and Neuro-DiO should be stored at –20°C (M). Keep in a closed container and protect from light.
Storage solution in DMSO can be stored 3-6 months at -20 degres Celsius.

Introduction

Carbocyanine dyes have hydrophilic/hydrophobic pattern, with strongest fluorescence when they are in membranes.



They are used in living and fixed tissues and cells. These dyes insert into the membrane, and diffuse rapidly, staining the entire cell surface.They allow the synaptic terminals tracing in a single motor unit.
DiI and DiO are also efficient postmortem neuronal tracers and used in neuroanatomy and visual science (Lukas 1998)

They can be combined together according to the spectre below, showing normalized fluorescence spectra in membranes.

DilC(3) and **DiOC(3)** are respectively compatible with rhodamine (TRITC) filter and fluorescein (FITC) filter.

$DiOC_1(3)$ is a fluorescent probe for measuring membrane potential.

$DiOC_{18}(3)$ [DiO] (3,3′ -dioctadecyloxacarbocyanine, perchlorate) is a widely used fluorescent membrane dye. However, DiO has been fluorescent emission and the lateral diffusion rate on the membranes is generally slower than that of DiI. DiO and DiI are often used together in dual color studies. Please also see our Neuro-DiO, which has improved property over DiO.

$DiOC_{14}(3)$ (3,3′ -ditetra decyloxacarbocyanine, hydroxyethanesulfonate) is a derivative of DiOC18(3) [DiO] but is more soluble in aqueous buffer. Staining is accomplished by simple incubation of cells in the buffer containing the dye.

SP-$DiOC_{18}(3)$ is a lipophilic sulfonated carbocyanine tracer probe.

$Di1C_{18}(3)$ [DiI] (1,1'-dioctadecyl-3,3,3' ,3'-tetramethylindocarbocyanine perchlorate) is a widely used carbocyanine membrane dye that labels cell membranes by inserting its two long (C_{18} carbon) hydrocarbon chains into the lipid bilayers. It is the most standard lipophilic dye for ER, Golgi studies.
Particularly, it has been extensively used for the anterograde and retrograde labeling of neurons. The intense fluorescence and high photostability of the dye make it possible to visualize the fine structures (axons and dendrites) of the neurons. Also, because of its low toxicity and the tendency to give highly stable cell labeling, the dye has been generally used for long term cell tracing of cells both in cultures and in living embryos or animals. The dye is usually applied to cells either from an ethanol solution (for cells in cultures) or directly from the dye crystals (for neurons in tissues, for example).
DiI emits its fluorescence in the orange red region and it can be used with standard fluoresceinand rhodamine optical filter, and combined to the green fluorescent dye DiO (FP-46805) for dual color studies.

$DiOC_6(3)$ is a cell-permeant, green-fluorescent, lipophilic dye that is selective for the mitochondria of live cells, when used at low concentrations. At higher concentrations, the dye may be used to stain other internal membranes, such as the endoplasmic reticulum.

Neuro-DiI and Neuro-DiO are derivatives respectively of Dil and DiO. They have better solubility and do not form aggregates, which tend to quench the fluorescence. Also, they diffuse faster than Dil and DiO on cell membranes and also may result in a more stable labeling.

$DilC_1$ (3), **$DiIC_5(3)$** and **$DilC_1(7)$** are potential-sensitive probes.

DiIC$_{18}$(5) [DiD] (1,1'--dioctadecyl-3,3,3',3'- tetramethylindodicarbocyanine, 4-chlorobenzenesulfonate salt) is similar to DiIC$_{18}$(3), but excitable with longer wavelength than carbocyanines (He-Ne laser). It is usefull when significant intrinsic fluorescence is observed with DiI or DiO

DiIC$_{18}$(7) [DiR] (1,1'-dioctadecyltetramethyl indotricarbocyanine Iodide) is lipophilic carbocyanine similar to DiI and DiO with near IR absorption and emission, allowing lowering the level of autofluorescence. It can be used in multicolor detection, combined to DiD (FP-22574A), DiI (FP-46804A) and Neuro-DiO (FP-AM330A).

6,6'-Ph2-DilC$_{18}$(3) is a cationic membrane probe.

DiA (4-(4-dihexadecylaminostyryl)-N-methylpyridinium iodide), a fluorescent carbocyanine dye, insert in membrane and commonly used for neuronal membrane tracing by diffusion (it diffuses faster than DiO). It is used in aldehyde-fixed tissue. It has very broad emission spectrum and can be detected with green, orange or even red filters. It is combined notably DilC18(3) for 2 colors staining.

Directions for use

Handling and Storage

Dialkylcarbocyanine dye is dissolved in DMF or ethanol at 1 mM or approximately 1mg/mL to make a stock solution.
These dyes are generally thermally stable. To facilitate the dissolution, the dyes can be put in a warm bath.
It is a good idea to filter the highly clark colored solution through a 0.2 or 0.45 µm membrane filter to ensure a clear solution. The solution thus prepared should be stored at room temperature and protect from light. To avoid dye re-precipitation, do not store the stock solution at below room temperature. The stock solutions must be examined for crystal formation. If crystals are noted, the solution should be warmed (at 37°C or a higher temperature) or sonicated to redissolve the crystals.

Some dyes are available in solution. It is used for microinjections in the place of crystalline dye.
The made solution in DMF is sonicated, centrifugated or filtrated to remove undissolved dye crystals.

Guidelines for use – on cells suspension

This procedure may serve as a reference for the use of following products : DiI, DiD, DiOC14(3), DiR, Neuro-DiI and Neuro-DiO. Optimization procedure may be necessary for each specific dye and cell type.
For optimal staining, prepare cells at a density of ~ 1 x 10^6/mL in a serum- free culture medium. If possible, use a single cell suspension for uniform cell staining. Divalent cations such as Ca^{2+} and Mg^{2+} may promote dye precipitation.
Therefore, for best result, we recommend the use of Dulbecco' s PBS (Ca^{2+} and Mg^{2+} free) for the staining.
Serum proteins and lipids should be removed from the medium because they may bind the dyes and reduce the effective dye concentration.

1- Add the dye stock solution to the cell suspension to achieve a final dye concentration of ~5 µM, or approximately 5 µg/uL, corresponding to a 200x dilution. Mix well by gentle pipeting.
2- Incubate at +37°C. Incubation time may vary from a few minutes to 20 min, depending on the cell types.
3- Separate the stained cells from the staining solution by centrifugation at +37 °C at 1500 rpm for a few minutes.
4- Remove the supernatant and resuspend the cells in fresh medium at +37 °C.
5- Wash the cells at least two more times according to steps 3 and 4.
6- Let the stained cells equilibrate for 10-15 min prior to fluorescence measurement.
7- Samples may be fixed with 2% paraformaldehyde and should be stable for up to 3 weeks.

Guidelines for use – on fixed tissue (Pavlidis, 2003)

1- Tissue is fixed in 4% paraformaldehyde in 0.1M phosphate buffer, pH7.4 at room temperature.
2- Incubation of the dye can be at +4°C or room temperature.

Note : higher temperature could be increase transcellular labeling.

FT-46804A

Permeabilizing reagents, detergents and high concentration of organic solvents may cause the degradation of labeling. Tissue stained with dye can be sectioned by cryostat or vibratome methods. But be careful to the possible bad resolution of Dil labeling.

Related products

- FP Membrane Markers (FPMM 1-43, 4-64, 2-10, 1-44, 1-84, 5-95; Synaptracer™)

References

Dil, DiO

- **Lukas JR,** *et al.*, «Carbocyanine Postmortem Neuronal Tracing: Influence of Different Parameters on Tracing Distance and Combination with Immunocytochemistry », *J. Histochem. Cytochem.,* **46,** 901 (1998) Article
- **Muñoz-Barroso** , *et al., «* Dilation of the Human Immunodeficiency Virus-1 Envelope Glycoprotein Fusion Pore Revealed by the Inhibitory Action of a Synthetic Peptide from gp41 », *J. Cell Biol.,* **140,** 315 (1998) Article
- **Soroceanu L**, *et al., «* Modulation of Glioma Cell Migration and Invasion Using Cl and K^+ Ion Channel Blockers », *J. Neurosci.,* **19**, 5942(1999) Article

Dilinoleyl DiI Solid

- **Chen C.-Y.** *et al*., Exercise Reduces GABA Synaptic Input onto Nucleus Tractus Solitarii Baroreceptor Second-Order Neurons via NK1 Receptor Internalization in Spontaneously Hypertensive Rats, *J. Neurosci*., 29: 2754 - 2761 (2009) Article
- **Savignat M.** *et al*., Rat Nerve Regeneration with the Use of a Polymeric Membrane Loaded with NGF, *Journal of Dental Research*, 86: 1051 - 1056 (2007) Article

$DiOC_1(3)$

- **Jacobberger JW** *et al*. Flow cytometric analysis of blood cells stained with the cyanine dye DiOC1[3]: reticulocyte quantification, *Cytometry*, 5(6):589-600 (1984) Abstract
- **Kelly T.M.** *et al*.: Photog. Sci.Eng. 18,68 (1974)

$DiOC_6(3)$

- **Doria M.** *et al.*, Protective function of autophagy during VLCFA-induced cytotoxicity in a neurodegenerative cell model, *Free Radical Biology and Medicine*, 137:46-58 (2019) Abstract
- **Saumet A.** *et al*.: Type 3-repeat/C-terminal domain of thrombospondin-1 triggers caspase-independent cell death through CD47/3 in promyelocytic leukemia NB4 cells**,** Blood **10**:1182 (2005)

".. was assessed using the 3,3'dihexyloxacarbocyanine iodide (DiOC 6 (3)) lipophilic cationic fluorochrome (20 nM, Interchim, Montlucon, France). ... "

$SP\text{-}DiOC_{18}(3)$

- **Demuth DR**, et al. Interaction of Actinobacillus actinomycetemcomitans outer membrane vesicles with HL60 cells does not require leukotoxin." *Cell Microbiol* 5, 111-21 (2003)
- **Zhang M, Kalinec F**. Distribution of Lipid-Soluble Fluorescent Dyes Reveal Domains in the Plasma Membrane of Guinea Pig Outer Hair Cells. *Mol Biol Cell* 9, 80a, abstract #461 (1998)

$DiOC_{18}(3)$ [DiO]

- **Bar D**.,*et al.*,« A continuous delivery system of IL-1 receptor antagonist reduces angiogenesis and inhibits tumor development », ***The FASEB Journal*** (2003) Article
- **Ellis R. E**., *et al.*, « The fog-3 Gene and Regulation of Cell Fate in the Germ Line of Caenorhabditis elegans », *Genetics*, **139**, 561(1995) Article

$DiIC_1(3)$

- **H.M.Shpiro.** Flow cytometric probes of early events in cell activation. *Cytomerty* 1, 301 (1981)

$DiIC_6(3)$

- **H.M.Shpiro.** Flow cytometric probes of early events in cell activation. *Cytomerty* 1, 301 (1981)

$DilC_{12}(3)$

- **Pfannkuche H**, *et al*. Intrinsic innervation patterns of the smooth muscle in the rumen and reticulum of lambs." *J Anat* 204, 293-9 (2004)
- **Wirth MJ** *et al*. Measurement and simulation of tailing zones of a cationic dye in analytical-scale reversed phase chromatography." *J Chromatogr* A 1034, 69-75 (2004)

FT-46804A

[DiI]

- **Pavlidis M.**, *et al.*, « Retinal Ganglion Cells Resistant to Advanced Glaucoma: A Postmortem Study of Human Retinas with the Carbocyanine Dye DiI », *Investigative Ophthalmology and Visual Science*., **44,** 5196 (2003) Article
- **Bernier PJ.**, *et al.*, « Newly generated neurons in the amygdala and adjoining cortex of adult primates », *PNAS*, **99**, 11464(2002) Article
- **Caulfield JP**, *et al.*, « Human erythrocytes adhering to schistosomula of Schistosoma mansoni lyse and fail to transfer membrane components to the parasite », *J. Cell Biol.,***101**, 158(1985) Article
- **Hannan A J**., « Characterization of nodular neuronal heterotopia in children », *Brain*, **122**, 219(1999) Article
- **Huesa G**, *et al.,* « Afferent and efferent connections of the cerebellum of the chondrostean Acipenser baeri : a carbocyanine dye (DiI) tracing study », *J Comp Neurol* **460**, 327 (2003) Abstract
- Kennedy A **L.,** ***et al.,*** « Duodenal Sensory Neurons Project to Sphincter of Oddi Ganglia in Guinea Pig », *The Journal of Neuroscience*, , **18**, 8065 (1998) **Article**
- **Lynch JM.**, *et al.*, « Increased Protection against Pneumococcal Disease by Mucosal Administration of Conjugate Vaccine plus Interleukin-12 », *Infection and Immunity*, **71** 4780, (2003) Article
- **Sund SE.,** *et al.*, « Cell Membrane Orientation Visualized by Polarized Total Internal Reflection Fluorescence », *Biophys J*, **77**, 2266 (1999) **Article**
- Zimmermann **,** ***et al.,*** « Lipoprotein Lipase Mediates the Uptake of Glycated LDL in Fibroblasts, Endothelial Cells, and Macrophages », ***Diabetes,*** **50**, 1643 (2001) **Article**

Δ^9-DiI

- **Worl J**, *et al*., Nonvagal origin of galanin-containing nerve terminals innervating striated muscle fibers of the rat esophagus. *Cell Tissue Res* 292, 453-461 (1998)
- **Cheng Z**, et al. FJ 3rd. *J Comp Neurol* 381, 1-17 (1997)

[DiI] and [DiR]

- **Derzko, Z**., *et al*., *Biochemistry,* **19**, 6050(1980)
- **Leuther, M.D**., *et al*., « Changes in lectin receptor lateral mobilities accompany lymphocyte stimulation », *J. Immunology*, **127**, 893(1981) Abstract
- **Honig, M..,** *et al.*, « Fluorescent carbocyanine dyes allow living neurons of identified origin to be studied in long-term cultures », *J. Cell Biol*., **103,** 171(1986) Article
- **Honig, M.G**., et al.,« Carbocyanine dyes. Novel markers for labelling neurons. », *Trends in Neurosci*., **9**, 333 (1989)
- **McConnell, S.K.**, *et al.*, « « Subplate neurons pioneer the first axon pathway from the cerebral cortex », *Science* **245**, 978(1989). Abstract

$DiOC_{14}(3)$ and [DiI]

- **Shahrokh Z**, *et al.,* « Distance between skeletal protein 4.1 and the erythrocyte membrane bilayer measured by resonance energy transfer », *J. Biol. Chem.,* **266**, 12082 (1991) Article

[DiD]

- **Lynch JM.**, *et al.*, « Increased Protection against Pneumococcal Disease by Mucosal Administration of Conjugate Vaccine plus Interleukin-12 », *Infection and Immunity*, **71** 4780, (2003) Article

[DiA]

- **Brudzynski S.M**., *et al.*, « Mesolimbic Component of the Ascending Cholinergic Pathways: Electrophysiological-Pharmacological Study », *J Neurophysiol*, **79**, 1675 (1998) Article
- **Garel S.**, *et al.*, « Molecular regionalization of the neocortex is disrupted in *Fgf8* hypomorphic mutants », *Development*, **130**, 1903(2003) Article
- **Shu T.,** *et al.,* « Slit2 Guides Both Precrossing and Postcrossing Callosal Axons at the Midline *In Vivo* », J. Neurosci., **23**, 8176(2003) Article

Ordering information

Catalog size quantites and prices may be found at http://www.fluoprobes.com
Please inquire for higher quantities (avaibility, shipment conditions).

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