FluoProbes®

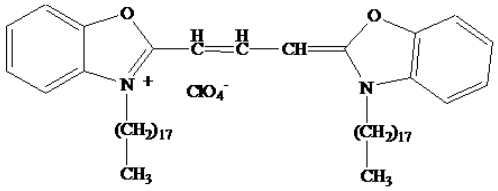
FT-46804A

Advion Interchim scientific

DiI, DiD, DiO, DiR, DiA, DiB

Lipophilic carbocyanine fluorescent dyes for membrane labeling

Product Information

cat.number	MW ($g \cdot mol^{-1}$) CAS #	$\lambda_{exc}\backslash\lambda_{em}$ 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, on demand	1115.55	498 / 514	175 000	MetOh / EtOH DMSO
5,5'-Ph2-$DiOC_{18}(3)$ (L) FP-M1610A, on demand	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-209202, 25 mg	432.25 36536-22-8	638/ 658		DMSO, DMF EtOH
$DiIC_1(7)$ (L) FP-C86280, on demand	509.04 16595-48-5	740 / 766		DMSO EtOH, $CHCl_3$
$DiIC_5(3)$ (L) FP-BT5040, on demand	596.63 53290-46-3	552 / 576		DMSO EtOH, DMF
$DiIC_{12}(3)$ (L) FP-467361, 25 mg	765.56 75664-01-6	550 / 566	144 000	DMSO, DMF EtOH, $CHCl_3$
$DiIC_{16}(3)$ (L) FP-467461, 25 mg	877.77 78566-75-3	548 / 566	148 000	DMSO EtOH

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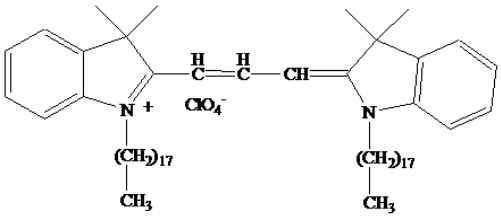
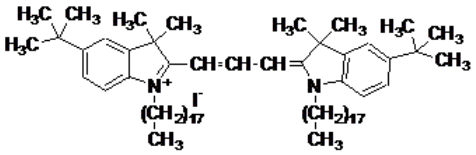
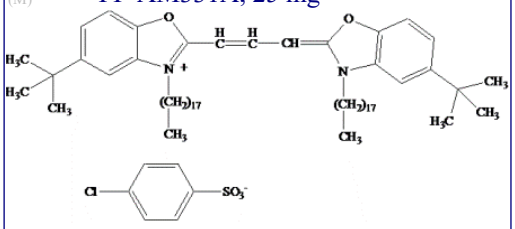
cat.number	MW ($g \cdot mol^{-1}$) CAS #	λ_{exc} \ λ_{em} max. (nm)	Mol. abs. ($M^{-1}cm^{-1}$)	Soluble in
DilC$_{18}$(3) [Dil] (*) (L) FP-46804A, 50 mg 	933.88 41085-99-8	551 / 566	148 000	DMSO EtOH, CH_3CN
DilC$_{18}$(3) [Dil], crystalline (L) FP-162451, 25 mg	933.88 41085-99-8	551 / 566	148 000	DMSO, DMF EtOH, $CHCl_3$
Dilinoleyl Dil Solid (L) FP-12792A, 5 mg	1017.97	549 / 564	134 600	DMSO
Neuro-Dil (L) FP-AM330A 25 mg 	1075.58	550 / 565	145 000 (MetOH)	DMSO / DMF MetOH / EtOH
Δ^9-Dil (L) FP-M1280A, 25 mg	925.49	550 / 565		DMSO, CH_3CN EtOH, DMF
5,5'-Ph2-DilC$_{18}$(3) (L) FP-WU4890, 5 mg	1022.09	576 / 598		DMSO, $CHCl_3$ EtOH, DMF
DilC$_{18}$(5) [DiD] 4-chlorobenzenesulfonate salt (M) FP-22574A, 50 mg	1052.1	644 / 663	193 000	DMSO EtOH, DMF
DilC$_{18}$(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
DilC$_{18}$(5) oil [DiD] iodide FP-DY3330, 25 mg	987.36 127274-91-3	644 / 665	270 000	DMSO
DilC$_{18}$(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

(*) DilC$_{18}$(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)

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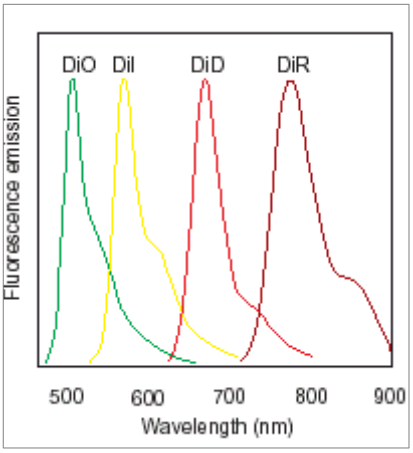
Storage: $DiOC_{18}(3)$, $DiOC_{14}(3)$, DiI, Neuro-DiI, Dilinoleyl DiI solid, DiA, DiB can be stored at +4°C (L)
DiD, DiR and Neuro-DiO should be stored at –20°C (M). Keep in a closed container and protect from light.
Storage solution in DMSO can be stored 3-6 months at -20 degres Celsius.

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.

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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.
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™)

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