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Product Information

Lipophilic Carbocyanine Dyes

Catalog no.	Product	Unit size	Ex/Em (nm)	MW
60010	DiI ($DiIC_{18}$(3))	50 mg	549/565	933.88
60011	DiO ($DiOC_{18}$(3))	50 mg	484/501	882
60012	$DiOC_{14}$(3), hydroxyethanesulfonate	50 mg	484/501	795
60013	DiA	50 mg	491/613	787
60014-10mg	DiD ($DiIC_{18}$(5))	10 mg	644/663	1052.1
60014-5mg	DiD ($DiIC_{18}$(5))	5 x 1 mg	644/663	1052.1
60015	Neuro-DiO	25 mg	484/501	1086
60016	Neuro-DiI	25 mg	549/565	1072
60017-10mg	DiR ($DiIC_{18}$(7))	10 mg	748/780	1013.4
60017-5mg	DiR ($DiIC_{18}$(7))	5 x 1 mg	748/780	1013.4
60018	DiI in Vegetable Oil	0.5 mL	549/565	1086
60019	Neuro-DiO in Vegetable Oil	200 uL	484/501	1086
60020	Neuro-DiI in Vegetable Oil	200 uL	549/565	1072
60034	Dilinoleyl DiI (FAST DiI™)	5 mg	549/565	925.82
60035	Dilinoleyl DiO (FAST DiO™)	5 mg	484/499	873.65
60036	DiB	10 mg	353/442	1074
60038	$DiOC_{16}$(3)	25 mg	484/501	921.30

Storage and Handling

Store dye solids at 4°C, protected from light.

Store vegetable oil dye solutions (60018, 60019, 60020) at room temperature, protected from light; do not freeze.

Products are stable for at least 1 year from date of receipt when stored as recommended.

Spectral Properties

See product table and Figure 1.

Preparing Dye Stock Solutions

Most lipophilic carbocyanine dyes are soluble in DMSO, DMF, or ethanol. For cell membrane labeling, we recommend preparing dye stock solutions in ethanol at 1-2 mM (~1-2 mg/mL). The dyes take time to dissolve, and you may need to heat the solution to 55°C with periodic vortexing to solubilize the dye.

DiI, Neuro-DiI, and Neuro-DiO also can be dissolved at 1-2 mM in vegetable oil by heating to 55°C and sonicating for 30 minutes or longer. Catalog numbers 60018, 60019, and 60020 are vegetable oil solutions, ready-to-use for microinjection.

DiO stock solutions can be prepared using one of the following methods; the dye may take an hour or more to dissolve in DMSO or DMSO:EtOH.

- 2 mM (1.76 mg/mL) in DMF with heating to 55°C and vortexing.
- 1 mM (0.88 mg/mL) in DMSO with heating to 55°C and periodic vortexing.
- 2 mM (1.76 mg/mL) in 1:1 DMSO:EtOH; add DMSO to the dye, vortex, then add an equal volume of EtOH, followed by heating to 55°C with periodic vortexing.

Dye stock solutions can be stored at 4°C, protected from light, for at least 12 months. If solutions become cloudy, re-heat to solubilize. See the protocols on the next page for information on preparing working solutions of dyes for staining.

Product Description

Lipophilic carbocyanine dyes are cyanine fluorescent dyes with hydrophobic hydrocarbon tails. The dyes are weakly fluorescent in aqueous phase, but become highly fluorescent in lipid bilayers, making them useful for labeling membranes. The dyes label cytoplasmic membrane and intracellular membrane structures in a wide variety of cell types efficiently and stably (1). They have been used as tracers in studies of cell fusion (2,3), cellular adhesion (4,5), and migration (6) due to their low cytotoxicity and high resistance to intercellular transfer. Cell populations can be labeled with different fluorescent colors for identification after mixing. Double labeling can identify cells that have fused or formed clusters (7). The dyes are also used for axonal tracing of neurons (8). Cells can be fixed with formaldehyde either before or after staining (see protocols on the next page).

Biotium offers a selection of carbocyanine dyes that vary in solubility, membrane diffusion, and fluorescence emission:

- DiI ($DiIC_{18}$(3; 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine) is a bright and photostable red fluorescent membrane dye widely used for membrane labeling, cell tracing, axonal tracing, and super-resolution imaging by STORM (9). The dye also is available in vegetable oil for microinjection studies.
- DiO ($DiOC_{18}$(3)) is a widely used green fluorescent membrane dye; the lateral diffusion rate on the membranes is generally slower than that of DiI.
- $DiOC_{14}$(3), hydroxyethanesulfonate is a DiO derivative with shorter hydrocarbon chains that is more soluble in aqueous media than DiO.
- DiA 4-(4-dihexadecylaminostyryl)-N-methylpyridinium iodide) is a yellow-green fluorescent membrane dye which diffuses much faster than DiO in cell membranes. DiA and DiI have been used together for two-color staining.
- DiD ($DiIC_{18}$(5); 1,1'-dioctadecyl-3,3,3',3'- tetramethylindodicarbocyanine, 4-chlorobenzenesulfonate salt) is a far-red fluorescent membrane dye that also has been used for super-resolution imaging by STORM (9).
- Neuro-DiO was developed at Biotium as a replacement for DiO, which has low solubility, a tendency to form non-fluorescent aggregates, and slow lateral diffusion rate. Neuro-DiO has better solubility in membranes and does not form aggregates. The dye also is available in vegetable oil for microinjection studies.
- Neuro-DiI was developed at Biotium as an alternative to the widely used DiI dye. Like Dilinoleyl DiI, Neuro-DiI has structural features that may make the probe diffuse faster than DiI on cell membranes. However, Neuro-DiI dye has saturated carbon chains, making it more hydrophobic than Dilinoleyl DiI, for potentially more stable labeling with less dye transfer between cells. Neuro-DiI also is available in vegetable oil for microinjection studies.
- DiR ($DiIC_{18}$(7); 1,1'-dioctadecyl-3,3,3',3'-tetramethylindotricarbocyanine iodide) is a near-infrared membrane dye that has been used for small animal *in vivo* imaging (10) and super-resolution imaging by STORM (9).
- Dilinoleyl DiI (1,1'-dilinoleyl-3,3,3',3'-tetramethylindocarbocynanine perchlorate) (also known as FAST DiI™) has unsaturated hydrocarbon chains for a much faster lateral diffusion rate in cell membranes than DiI, making it particularly useful for tracing neurons in tissues.
- Dilinoleyl DiO (1,1'-dilinoleyl-3,3'-oxacarbocynanine perchlorate) (also known as FAST DiO™) has unsaturated hydrocarbon chains for a much faster lateral diffusion rate in cell membranes than DiO.
- DiB is Biotium's unique blue fluorescent carbocyanine membrane dye. **Note:** For labeling cells with DiB, we recommend using our CellBrite™ Blue Cytoplasmic Membrane Labeling Kit (30024, see Related Products), which includes a cell loading buffer that is required for efficient cell staining.
- $DiOC_{16}$(3) is a green fluorescent lipophilic cyanine dye with two C_{16} hydrocarbon chains that can be used to stain cytoplasmic membranes.

References

1. J Cell Biol 103, 171 (1986); 2. J Cell Biol 135, 63 (1996); 3. Cytometry 21, 160 (1995); 4. J Biol Chem 273, 33354 (1998); 5. J Cell Biol 136, 1109 (1997); 6. Anticancer Res 18, 4181 (1998); 7. J Immunol Methods 156, 179 (1992); 8. Trends Neurosci 12(9):333 (1989); 9. Proc Natl Acad Sci U S A (2012) 109:13978-13983; 10. Curr Drug Deliv. 2016;13(1):40-8; 11. J Neurosci Methods 174, 71 (2008).

Cell Staining Protocols

You may need to optimize the staining procedure for each particular cell type by varying the dye concentration, staining volume, labeling time, and/or wash steps.

Note: For labeling cells with DiB, we recommend using our CellBrite™ Blue Cytoplasmic Membrane Labeling Kit (30024, see Related Products), which includes a cell loading buffer that is required for efficient cell staining.

Labeling Live Cells in Suspension

1. Suspend cells at a density of 1×10^6/mL in normal growth medium.
2. Add carbocyanine dye stock solution to 1 mL of cell suspension at a final concentration of 1-10 uM. Mix well by low-speed vortexing or flicking the tube.
3. Incubate for 20 minutes at 37°C. The optimal incubation time will vary depending on cell type. Start with 20 minutes and optimize as needed for uniform labeling.
4. Pellet the cells by centrifugation at 350 x g for 5 minutes.
5. Remove the supernatant and wash the cells by gently resuspending them in warm (37°C) medium.
6. Repeat the centrifugation and wash steps (Steps 4 and 5) two more times.
7. Image fluorescence. Cells can be imaged in culture medium.

Labeling Live Adherent Cells

1. Prepare staining medium by adding carbocyanine dye stock solution to normal growth medium at a final concentration of 1-10 uM and mixing well.
2. Remove growth medium from the cells.
3. Add enough staining medium to completely cover the cells.
4. Incubate the cells at 37°C. The optimal incubation time will vary depending on the cell type. Start with 20 minutes and optimize as needed for uniform labeling.
5. Remove the staining medium.
6. Wash the cells by adding fresh warm growth medium and incubating at 37°C for 5 minutes. Repeat this wash step two more times.
7. Image fluorescence. Cells can be imaged in culture medium.

Long Term Cell Staining

Carbocyanine dye staining has been reported to be retained by live cells for weeks in culture or *in vivo*. Immediately after labeling cells, the dyes primarily stain the plasma membrane, even in fixed cells. However, if live cells are cultured after staining, the labeled membrane will be internalized in intracellular vesicles, usually becoming mostly intracellular after several hours in commonly used cell lines.

Fixation After Staining

Cells stained with carbocyanine dyes can be fixed with formaldehyde (PFA), but not methanol or other solvents. Staining can withstand permeabilization with 0.1% Triton® X-100 or 0.1% digitonin (11), but permeabilization can cause increased intracellular staining. Alternatively, we have seen good preservation of plasma membrane staining when cells are fixed with formaldehyde, then permeabilized before staining (see Labeling Fixed Cells). Also see our CellBrite™ Fix and MemBrite™ Fix Stains under Related Products. These stains covalently label cell membranes or cell surface proteins for truly fixable staining.

Labeling Fixed Cells

Note: Cells should be fixed with formaldehyde (PFA). Fixation with methanol or other solvents will extract cellular lipids and cause poor staining.

1. Wash cells with PBS after fixation.
2. Optional: Permeabilize cells with 0.1% Triton® X-100 in PBS or Biotium's Permeabilization Buffer for 10 minutes at room temperature.
   Note: We have found this method to preserve plasma membrane staining better than permeabilization with digitonin or saponin.
3. Wash the cells 3 times with PBS to remove all traces of detergent.
4. Optional: Perform staining with antibodies or other dyes. Do not use detergent in the buffers used for blocking, antibody dilution, or washing.
5. Prepare staining solution: Add carbocyanine dye stock solution to PBS or other buffer at a final concentration of 1-10 uM and mix well.
6. Remove the buffer from the cells and add the staining solution.
7. Incubate 10 minutes or longer at room temperature, in the dark.
8. Wash the cells 3 times with PBS.
9. Image in PBS. Do not use mounting medium (see Mounting Samples for Imaging below).

Mounting Samples for Imaging

Do not use mounting medium with carbocyanine dyes; glycerol or organic solvents in mounting media will solubilize the dyes, resulting in increased intracellular staining and high background over time. We recommend imaging in directly in PBS (or other aqueous buffers). Coverslips should be mounted using PBS and sealed with a suitable coverslip sealant such as CoverGrip™ or nail polish. Stained samples can be stored in PBS at 4°C for several weeks or longer.

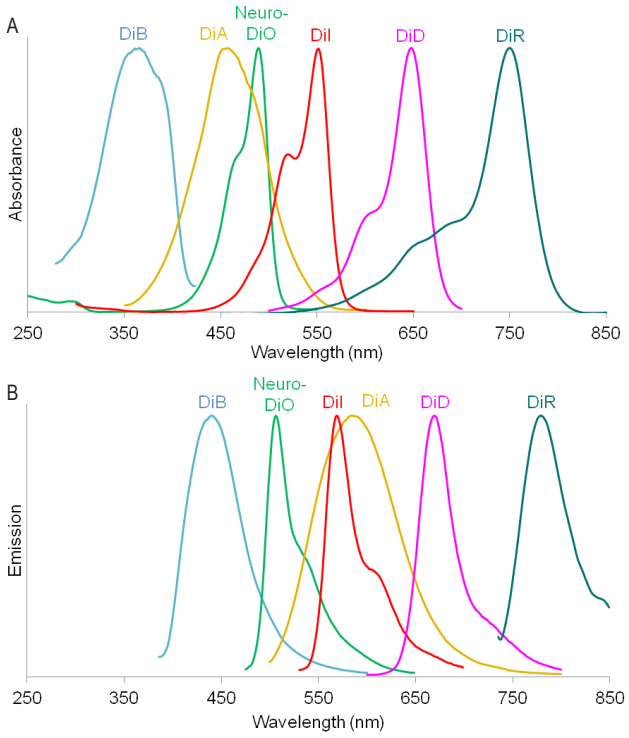

Figure 1. Representative absorbance (A, top) and emission (B, bottom) spectra for lipophilic carbocyanine dyes in methanol. DiO, Dilinoleyl DiO, $DiOC_{14}(3)$, and $DiOC_{16}(3)$ are spectrally similar to Neuro-DiO; Neuro-DiI and Dilinoleyl DiI are spectrally similar to DiI.

Related Products

Catalog number	Product
30024	CellBrite™ Blue Cytoplasmic Membrane Labeling Kit
30021	CellBrite™ Green Cytoplasmic Membrane Dye
30022	CellBrite™ Orange Cytoplasmic Membrane Dye
30023	CellBrite™ Red Cytoplasmic Membrane Dye
30070-30079	CellBrite™ NIR Near-Infrared Cytoplasmic Membrane Dyes
30090	CellBrite™ Fix 488 Fixable Membrane Stain
30088	CellBrite™ Fix 555 Fixable Membrane Stain
30089	CellBrite™ Fix 640 Fixable Membrane Stain
30092-30099	MemBrite™ Fix Fixable Cell Surface Staining Kits
30101-30104	MemBrite™ Fix-ST Fixable Cell Surface Stains for STORM
70065	LipidSpot™ 488 Lipid Droplet Stain
70069	LipidSpot™ 610 Lipid Droplet Stain
40083	NucSpot® 470 Nuclear Stain for dead or fixed cells
40081	NucSpot® Live 488 Nuclear Stain for live or fixed cells
40082	NucSpot® Live 650 Nuclear Stain for live or fixed cells
40060	RedDot™1 Far-Red Nuclear Stain for live cells
40061	RedDot™2 Far-Red Nuclear Stain for dead or fixed cells
22020	10X Phosphate Buffered Saline (PBS)
22023	Paraformaldehyde, 4% in PBS, Ready-to-Use Fixative
22016	Permeabilization Buffer
23005	CoverGrip™ Coverslip Sealant

Please visit our website at www.biotium.com for information on our life science research products, including nuclear, organelle, and apoptosis stains for live cell and real-time imaging, fluorescent CF® dye antibodies and other conjugates, and other fluorescent probes, reagents, and kits for cell biology research.