# DiO

**Cat. No.:** HY-D0969

**CAS No.:** 34215-57-1

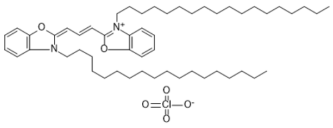
**Molecular Formula:** $C_{53}H_{85}ClN_2O_6$

**Molecular Weight:** 881.7

**Target:** Fluorescent Dye

**Pathway:** Others

**Storage:** 4°C, sealed storage, away from moisture and light
* In solvent : -80°C, 6 months; -20°C, 1 month (sealed storage, away from moisture and light)



## SOLVENT & SOLUBILITY

**In Vitro**

DMF : 10 mg/mL (11.34 mM; Need ultrasonic)
DMSO : 5 mg/mL (5.67 mM; ultrasonic and warming and heat to 60°C)

| Preparing Stock Solutions | Mass / Solvent / Concentration | 1 mg | 5 mg | 10 mg |
|---|---|---|---|---|
| | 1 mM | 1.1342 mL | 5.6709 mL | 11.3417 mL |
| | 5 mM | 0.2268 mL | 1.1342 mL | 2.2683 mL |
| | 10 mM | 0.1134 mL | 0.5671 mL | 1.1342 mL |

Please refer to the solubility information to select the appropriate solvent.

## BIOLOGICAL ACTIVITY

**Description**

DiO is a long-chain carbocyanine dye. Carbocyanine dyes are widely used as Di to label cells, organelles, liposomes, viruses and lipoproteins[2].

**In Vitro**

Carbocyanine dyes are widely used as Di to label cells, organelles, liposomes, viruses and lipoproteins. Long-chain carbocyanines which include DiO (DiOC18(3)), DiI (DiIC18(3)), DiD (DiIC18(5)) and DiR, and dialkyl aminostyryl dye DiA (4-Di-16-ASP) are used for labeling membranes and other hydrophobic structures. DiIC16(3) has shorter alkyl substituents (C16) than DiI (C18). They have extremely high extinction coefficients, environmental dependent fluorescence and short excited-state lifetimes in lipid environments. They are oils at room temperature and weakly fluorescent in water but highly fluorescent and quite photostable when incorporated into membranes or bound to lipophilic biomolecules. These optical characteristics make them ideal for staining the cytoplasmic membranes of cells. Once applied to cells, these dyes diffuse laterally within the plasma membrane, resulting in staining of the entire cell[1].
DiO, DiI, DiD and DiR exhibit distinct green, orange, red and infrared fluorescence, respectively thus facilitating multicolor imaging and flow cytometric analysis of live cells . DiO and DiI can be used with standard FITC and TRITC filters respectively. Among them DiI and its analogs are most frequently used since they usually exhibit very low cell toxicity. In addition, DiI is widely used for determining lipoproteins such as LDL and HDL. The lipophilic aminostyryl dye DiA is also often used for

neuronal tracing[2].

General Protocol

1. Preparing Stain Solutions of Di

a. Prepare DMF, DMSO or ethanol stock solutions: The stock solutions should be prepared in dimethyl formamide (DMF), dimethylsulfoxide (DMSO, or ethanol DMSO at 1-5 mM. DMF is preferable to ethanol as a solvent for Di. The stock solution should be used promptly. Any unused solution need to be aliquoted and refrozen at least -20℃. Avoid repeated freeze/thaw cycle. The solution can be stored for 6 months.

b. Prepare working solutions: Dilute the stock solutions into a suitable buffer such as serum-free culture medium, HBSS or PBS to make 1 to 5 μM working solutions. We do not recommend storing the aqueous solution for more than one day.

Note: The final concentration of the working solution should be empirically determined for different cell types and/or experimental conditions.

2. Suspension cells

a. Centrifuge at 1000 g at 4℃ for 3-5 minutes and then discard the supernatant. Wash twice with PBS, 5 minutes each time.The cell density is $1\times10^6$/mL.

b. Add 1 mL of Di working solution, and then incubate at room temperature for 5-30 minutes.

c. Centrifuge at 400 g at 4℃ for 3-4 minutes and then discard the supernatant.

d. Wash twice with PBS, 5 minutes each time.

e. Resuspend cells with serum-free cell culture medium or PBS.Observation by fluorescence microscopy or flow cytometry.

3. Adherent cells

a. Culture adherent cells on sterile coverslips.

b. Remove the coverslip from the medium and aspirate excess medium.

c. Add 100 μL of working solution, gently shake it to completely cover the cells, and then incubate at room temperature for 5-30 minutes.

d. Wash twice with medium, 5 minutes each time. Observation by fluorescence microscopy or flow cytometry.



## CUSTOMER VALIDATION

- ACS Nano. 2023 Apr 18.
- Biomater Res. 2023 Apr 15;27(1):30.
- Anal Chem. 2023 Sep 7.
- J Transl Med. 2022 Jul 6;20(1):307.
- PLoS Pathog. 2022 Oct 12;18(10):e1010907.

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## REFERENCES

[1]. Gan WB, et al. Multicolor "DiOlistic" labeling of the nervous system using lipophilic dye combinations. Neuron. 2000 Aug;27(2):219-25.

[2]. Bhowmik BB, et al. Photophysical studies of 3,3' dioctadecyloxacarbocyanine dye in model biological membranes and different solvents. Chem Phys Lipids. 2001 Feb;109(2):175-83.

[3]. Warren GL, et al. Redistribution of cell membrane probes following contraction-induced injury of mouse soleus muscle.

[4]. Bhowmik BB, et al. Photophysical studies of 3,3' dioctadecyloxacarbocyanine dye in model biological membranes and different solvents. Chem Phys Lipids. 2001 Feb;109(2):175-83.

**Caution: Product has not been fully validated for medical applications. For research use only.**

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