

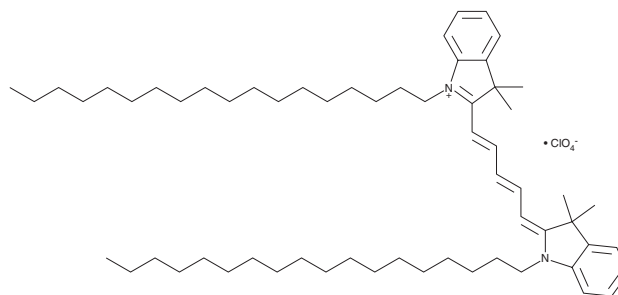
PRODUCT INFORMATION



DiD (perchlorate)

Item No. 45033

CAS Registry No.: 127274-91-3
Formal Name: 2-[5-(1,3-dihydro-3,3-dimethyl-1-octadecyl-2H-indol-2-ylidene)-1,3-pentadien-1-yl]-3,3-dimethyl-1-octadecyl-3H-indolium, perchlorate
MF: $C_{61}H_{99}N_2 \cdot ClO_4$
FW: 959.9
Purity: $\geq 98\%$
Ex./Em. Max.: 644/663 nm
Supplied as: A solid
Storage: $-20^\circ C$
Stability: ≥ 4 years
Special Conditions: Light and moisture sensitive



Information represents the product specifications. Batch specific analytical results are provided on each certificate of analysis.

Laboratory Procedures

DiD (perchlorate) is supplied as a solid. A stock solution may be made by dissolving the DiD (perchlorate) in the solvent of choice, which should be purged with an inert gas. DiD (perchlorate) is slightly soluble (0.1-1 mg/ml) in chloroform:methanol (1:1) and ethanol.

Description

DiD is a fluorescent probe for cell membranes and other hydrophobic structures. It displays excitation/emission maxima of 644/663 nm, respectively, with an increase in fluorescence intensity when incorporated into membranes or bound to lipophilic biomolecules. DiD can be used in conjunction with additional probes for multi-labeling applications and in flow cytometric analysis of live cells.

Assay Protocol

1. Prepare membrane-staining solution

- Dissolve DiD in ethanol to a final concentration of 1-5 mM to make the membrane staining stock solution.
- Dilute the membrane staining stock solution in serum-free culture medium or a suitable buffer, such as HBSS or PBS, to make a 1-5 μM dye working solution

NOTE 1: Aliquot and store unused DiD stock solution at $<-20^\circ C$. Repeated freeze-thaw cycles should be avoided.

NOTE 2: The final concentration of the working solution is application-specific. Staining conditions may be modified according to cell type and/or experimental conditions. It is recommended to test at concentrations at least over a ten-fold range.

2. Prepare and stain the cells

- Adherent cells:**
 - Grow cells on sterile glass coverslips.
 - Remove coverslips from growth medium and drain off any excess medium. Place coverslips in a humidity chamber.
 - Add 100 μl of the dye working solution prepared in step 1B to the corner of the coverslip and gently agitate until all cells are covered.

WARNING

THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

SAFETY DATA

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the [complete](#) Safety Data Sheet, which has been sent via email to your institution.

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- iv. Incubate cells at 37°C, 5% CO₂ for 2-20 minutes.*
- v. Drain off the dye working solution and wash the coverslips 2-3 times with growth medium. For each wash cycle, cover the cells with pre-warmed growth medium, incubate for 5-10 minutes, and then drain off the medium.
- vi. Observe cells using the fluorescence technique of choice.

B. Suspension cells:

- i. Suspend cells at a density of 1×10^6 per ml in dye working solution.
- ii. Incubate cells at 37°C for 2-20 minutes.*
- iii. Centrifuge the labeled suspension cells at 1,000-1,500 rpm for 5 minutes.
- iv. Remove the supernatant and gently resuspend the cells in pre-warmed growth medium.
- v. Repeat steps iii. and iv. two more times.
- vi. Observe cells using the fluorescence technique of choice.

**NOTE 3: The optimal incubation time varies depending on cell type. It is recommended to start with a 20-minute incubation and optimize as needed to obtain uniform labeling.*

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