# Product Information

## DiOC2(3) (3, 3'-Diethyloxacarbocyanine Iodide)

Catalog Number: D4027

Product Size: 20 mg

Application Scope: Membrane potential staining

## Parameters

Appearance: Orange-red solid soluble in DMSO or DMF

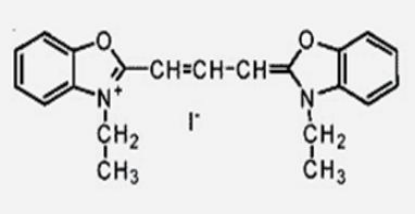
Ex/Em (MeOH): 482/497 nm

CAS No.: 905-96-4

Molecular Formula: $C_{21}H_{21}IN_2O_2$

Molecular Weight: 460.31

Molecular Structure:



## Storage

Store at -20℃ and protect from light. When stored as directed, product is stable for at least 12 months.

## Description

DiOC2(3) has been used for measuring membrane potentials in bacteria. DiOC2(3) penetrates the cytosol of eukaryotic cells. At concentrations below 100 nM, the dye accumulates primarily in mitochondria with active membrane potentials, and red emission increases due to dye stacking. DiOC2(3) stain intensity decreases when cells are treated with reagents that disrupt mitochondrial membrane potential.

Cells stained with DiOC2(3) can be visualized by flow cytometry with blue excitation and green and red emissions. The reagent can be paired with other reagents, such as Annexin V–APC, for multiparametric study of vitality and apoptosis.

## Protocol

**Labeling Cells with DiOC2(3)**

Before beginning the experiment, ensure that the vials of DiOC2(3) and CCCP have equilibrated to room temperature.

1.1 Preparation of stock solution: DiOC2 (3) was dissolved in DMSO to prepare a certain concentration of stock solution.

1.2 For each sample, suspend cells in 1 mL warm medium, PBS, or other buffer at approximately $1 \times 10^6$ cells/mL.

1.3 For the control tube, add 1 µL of 50 mM CCCP (50 µM final concentration) and incubate the cells at 37°C for 5 minutes.

Note: CCCP can be added simultaneously with DiOC2(3). Titration of the CCCP may be required for optimal results with each cell type.

1.4 Add amount of DiOC2(3) stock solution (50 nM final concentration) and incubate the cells at 37°C, 5% $CO_2$, for 15 to 30 minutes. If performing additional labeling, for example with an annexin V conjugate, follow the protocol below, beginning with step 2.1. If no additional staining is to be performed, proceed with the following steps.

1.5 OPTIONAL: Wash cells once by adding 2 mL of warm PBS or other buffer to each tube of cells.

1.6 Pellet the cells by centrifugation.

1.7 Resuspend by gently flicking the tubes. Add 500 µL PBS



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(or other suitable buffer) to each tube.

1.8 Analyze on a flow cytometer with 488 nm excitation. Using the CCCP-treated sample, perform standard compensation.

**Additional Labeling with an Annexin V Conjugate**

It is possible to label the DiOC2(3)-stained cells with other markers for apoptosis or viability. The example below is a protocol for labeling with Annexin V–APC:

2.1 After step 1.4 (above), wash cells once by adding 2 mL of warm PBS or other buffer to each tube of cells.

2.2 Pellet the DiOC2(3)-stained cells and resuspend in 100 µL of 1× annexin binding buffer (10 mM HEPES, 140 mM NaCl, and 2.5 mM $CaCl_2$, pH 7.4).

2.3 Add 5 µL annexin V conjugate (e.g. Annexin V–APC).

2.4 Incubate the samples at 37°C for 15 minutes.

2.5 Add 400 µL annexin binding buffer.

2.6 Analyze on a flow cytometer with 488 nm and 633 nm excitation using emission filters appropriate for YF488/PI and YF633 dye.

## Notes

1. There are quenching problems with fluorescent dyes. Please avoid light to slow down the fluorescence quenching.

2. For your safety and health, please wear lab coats and disposable gloves.



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