MitoProbe™ DiOC$_2$(3) Assay Kit for Flow Cytometry (M34150)

Quick Facts

Storage upon receipt:

- 2–6°C
- Protect from light
- Do not freeze

Ex/Em: 482/497 nm

Number of Assays: 100, based on labeling volumes of 1.0 mL

Introduction

Cationic cyanine dyes have been shown to accumulate in cells in response to membrane potential [1] and membrane potential changes have been studied in association with apoptosis.[2-3] The MitoProbe™ DiOC$_2$(3) Assay Kit provides solutions of the cyanine dye DiOC$_2$(3) (3,3′-diethyloxacarbocyanine iodide, Figure 1) and CCCP (carbonyl cyanide 3-chlorophenylhydrazone), for the study of mitochondrial membrane potential. DiOC$_2$(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. DiOC$_2$(3) stain intensity decreases when cells are treated with reagents that disrupt mitochondrial membrane potential, such as CCCP (Figure 2).

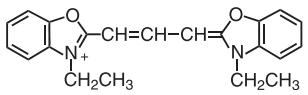

Figure 1. Structure of DiOC$_2$(3), molecular weight: 460.31.

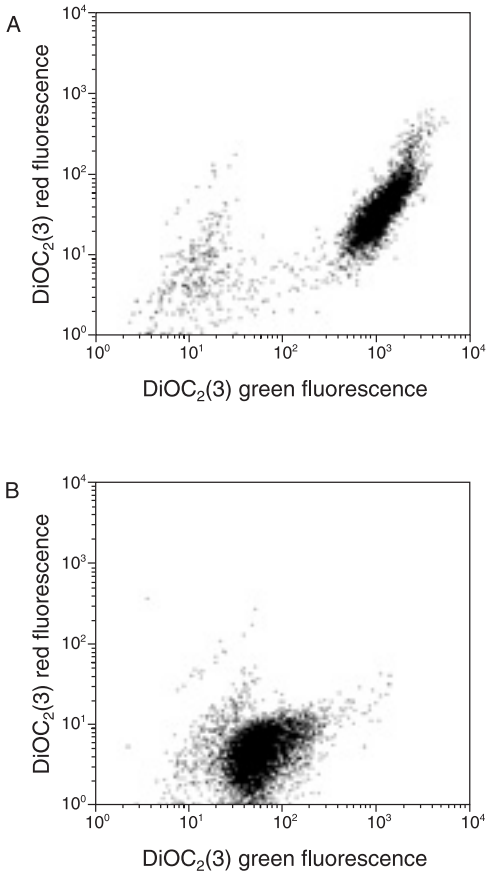

Figure 2. Jurkat cell response to DiOC$_2$(3). Either untreated (A) or CCCP-treated (B) Jurkat cells were stained with 50 nM DiOC$_2$(3). Cells were analyzed on a flow cytometer using 488 nm excitation and 530/30 nm bandpass and 650 nm longpass filters.

Cells stained with DiOC$_2$(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 red-excited annexin V allophycocyanin (A35110), for multiparametric study of vitality and apoptosis (Figure 3). Combining DiOC$_2$(3) dye with an annexin V conjugate results in superior resolution of subpopulations when compared to results obtained from other commonly used dyes. Using a ratiometric parameter (e.g. red/green intensity) appears to correct for size differences when staining bacteria [4-5] and isolated mitochondria. Red and green signals from intact cells stained with DiOC$_2$(3) appear to increase proportionally.

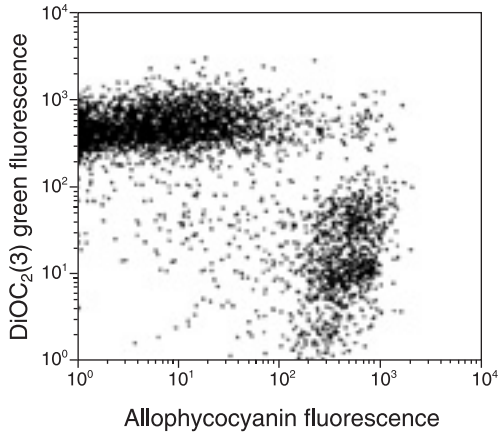

Figure 3. Camptothecin-treated Jurkat cells stained with $DiOC_2(3)$ and annexin V–allophycocyanin. Cells were incubated for 4 hours with camptothecin at 37°C, 5% CO_2, then stained with 50 nM $DiOC_2(3)$ and annexin V–allophycocyanin. Cells were analyzed on a flow cytometer using 488 nm and 633 nm excitations with 530/30 nm and 660/20 nm bandpass emission filters.

Materials

Kit Contents

- $DiOC_2(3)$, 625 µL of 10 µM $DiOC_2(3)$ in DMSO
- CCCP, 125 µL of 50 mM CCCP in DMSO

Storage and Handling

Upon receipt, components should be stored at 2–6°C. DO NOT FREEZE. Before opening, each vial must be at room temperature. When stored properly, both the $DiOC_2(3)$ and CCCP solutions should be stable for at least twelve months.

Spectral Characteristics

The approximate excitation and emission maxima of $DiOC_2(3)$ are 482 nm and 497 nm, respectively. Cells labeled with $DiOC_2(3)$ can be analyzed by flow cytometry using 488 nm excitation and green or red emission, and by fluorescence microscopy using standard filters for Alexa Fluor® 488 dye and Texas Red® dye.

Experimental Protocol

The following protocol describes introducing $DiOC_2(3)$ reagent into the cultured cells and analyzing the stained cells by flow cytometry. Suggested initial conditions may require modifications because of differences in cell types and culture conditions. The concentration of probe for optimal staining will vary depending upon the application. A concentration range should be tested, starting around 50 nM $DiOC_2(3)$. CCCP controls should be used to confirm that the $DiOC_2(3)$ response is sensitive to changes in membrane potential.

Labeling Cells with $DiOC_2(3)$

Before beginning the experiment, ensure that the vials of $DiOC_2(3)$ and CCCP have equilibrated to room temperature.

1.1 For each sample, suspend cells in 1 mL warm medium, phosphate-buffered saline, or other buffer at approximately 1×10^6 cells/mL.

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

Note: CCCP can be added simultaneously with $DiOC_2(3)$. Titration of the CCCP may be required for optimal results with each cell type.

1.3 Add 5 µL of 10 µM $DiOC_2(3)$ (supplied with the kit, 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 step 1.4.

1.4 OPTIONAL: Wash cells once by adding 2 mL of warm phosphate-buffered saline (PBS) or other buffer to each tube of cells.

1.5 Pellet the cells by centrifugation.

1.6 Resuspend by gently flicking the tubes. Add 500 µL PBS (or other suitable buffer) to each tube.

1.7 Analyze on a flow cytometer with 488 nm excitation using emission filters appropriate for Alexa Fluor® 488 dye and Texas Red® dye (Figure 2). Gate on the cells, excluding debris. Using the CCCP-treated sample, perform standard compensation.

Additional Labeling with an Annexin V Conjugate

It is possible to label the $DiOC_2(3)$-stained cells with other markers for apoptosis or viability, as long as the fluorescence emission of the additional label is spectrally resolved from $DiOC_2(3)$. The example below is a protocol for labeling with annexin V–allophycocyanin.

2.1 After step 1.3 (above), wash cells once by adding 2 mL of warm phosphate-buffered saline or other buffer to each tube of cells.

2.2 Pellet the $DiOC_2(3)$-stained cells and resuspend in 100 µL of 1X 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-allophycocyanin, A35110).

Note: 5 µL is appropriate for annexin V conjugates from Molecular Probes. Conjugates purchased from other suppliers may require a different volume to be effective.

2.4 Incubate the samples at 37°C for 15 minutes. (37°C is important to maintain membrane potential.)

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 Alexa Fluor® 488 dye/propidium iodide and Alexa Fluor® 633 dye (Figure 3).

References

1. Proc Natl Acad Sci U S A 76, 5728 (1979); **2.** Meth Cell Biol 63, 467 (2001); **3.** Exp Cell Res 214, 323 (1994); **4.** Methods 21, 271 (2000); **5.** Cytometry 35, 55 (1999).

Product List *Current prices may be obtained from our Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
M34150	MitoProbe™ $DiOC_2(3)$ *for flow cytometry* *100 assays*	1 kit

Contact Information

Further information on Molecular Probes products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Paisley, United Kingdom. All others should contact our Technical Service Department in Eugene, Oregon.

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