Cardiolipin Green Fluorescence Staining Kit (NAO)

Introduction

Cardiolipin is a phospholipid mainly located in the inner membrane of eukaryotic mitochondria and some Gram-negative bacteria. It maintains membrane integrity and supports energy metabolism as well as mitochondrial dynamics. Mitochondrial injury, apoptosis or metabolic disorders trigger early changes in cardiolipin content and localization. Thus cardiolipin detection is widely used to assess apoptosis, mitochondrial damage and metabolic status in cell biology, microbiology and mitochondrial-related disease research.

This kit uses 10-N-nonylacridine orange (NAO), a live-cell permeable fluorescent probe that specifically binds negatively-charged cardiolipin. NAO shows bright-green fluorescence at Ex/Em=498/521 nm; local oligomer stacking with cardiolipin may cause fluorescence red-shift. It can be applied for live-cell mitochondrial tracking, apoptosis-related cardiolipin monitoring, flow cytometry-based mitochondrial quality analysis and Gram-negative bacterial cardiolipin staining.

Components and Storage

Components \ Size	100 Assays	500 Assays	Storage
NAO (1000X)	0.1 mL	0.5 mL	-20°C away from light
Hoechst 33342 (1000X)	0.1 mL	0.5 mL	-20°C away from light
Staining Buffer	100 mL	500 mL	-20°C
Shipping: Blue ice	**Shelf life:** 1 year		

Protocol

1. Preparation of NAO staining working solution: Prepare appropriate volume of NAO staining working solution for 6 well plates according to the table below. Prepare working solution freshly before use.

	1 sample	10 samples	100 samples
NAO (1000X)	1 μL	10 μL	100 μL
Hoechst 33342 (1000X)	1 μL	10 μL	100 μL
Staining Buffer	998 μL	9.98 mL	99.8 mL

***Note:**

a) Keep away from light during preparation of working solution.

b) Cell culture medium may substitute for Staining Buffer. For purified mitochondria, mitochondrial storage buffer can be used instead.

c) NAO concentration in working solution may be adjusted for specific experiments. Optimal staining conditions are generally explored within the range of 0.5-2X working concentration.

2. Staining of suspension cells

1) Treat cells according to experimental design.

2) Harvest an appropriate amount of cells; centrifuge at 600 × *g* for 5 min at room temperature. Discard supernatant and add adequate NAO staining working solution to adjust cell density to 1 × 10^6 cells/mL.

3) Incubate for 15-45 min in a 37°C cell incubator. Incubation time varies across cell types; start with 15 min and further optimize as needed.

4) After incubation, centrifuge at 600 × *g* for 5 min at room temperature. Gently discard supernatant without disturbing cell pellets.

5) Wash cells twice with pre warmed (37°C) cell culture medium.

6) Resuspend cells in proper volume of cell culture medium. Samples can then be analyzed by fluorescence microplate reader, fluorescence microscope or flow cytometer.

NAO emits green fluorescence: Ex/Em = 498/521 nm;

Hoechst 33342 emits blue fluorescence: Ex/Em = 346/460 nm.

3. Staining of adherent cells

If adherent cells are to be analyzed by flow cytometry, harvest cells first and follow the protocol for suspension cells. For fluorescence microplate reader assays, black 96 well plates are recommended.

1) Treat cells according to experimental design.

2) For 6 well plates, discard culture medium; wash cells once with suitable buffer such as PBS if necessary.

3) Add sufficient NAO staining working solution to cover cells. For 6 well plates, add 1 mL working solution per well. Adjust volume for other plate formats accordingly.

4) Incubate for 15-45 min in a 37°C cell incubator. Incubation time varies across cell types; start with 15 min and further optimize as needed.

5) Upon completion of incubation, carefully aspirate working solution. Wash cells twice with pre warmed (37°C) cell culture medium.

6) Add appropriate volume of cell culture medium, then observe under a fluorescence microscope.

NAO emits green fluorescence: Ex/Em = 498/521 nm;

Hoechst 33342 emits blue fluorescence: Ex/Em = 346/460 nm.

4. Staining of purified mitochondria

1) For purified mitochondria, prepare NAO staining working solution with mitochondrial storage buffer.

2) Add 0.9 mL NAO staining working solution to 0.1 mL purified mitochondria (10-100 μg total protein), then mix thoroughly.

3) Refer to cell staining protocols and adjust incubation conditions appropriately.

4) Analyze stained samples by fluorescence microscope or fluorescence microplate reader.

NAO emits green fluorescence: Ex/Em = 498/521 nm.

Note

1. Fluorescent dyes are susceptible to photobleaching. Protect reagents and samples from light during handling and storage.
2. This kit is not suitable for staining fixed or frozen cells, tissues or purified mitochondria.
3. For your safety and health, please wear lab coats and gloves during the experiment.
4. For research use only. Not to be used in clinical diagnostic or clinical trials.

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