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Product Information

JC-9 Mitochondrial Potential Probe

Catalog Number	Product Name	Unit Size
C046	JC-9 Dye	5 mg

Storage upon receipt:
- -20°C
- Protect from light

Ex/Em = 514/529 nm, monomer form;
585/590 nm, J-aggregate form.

Product Description

JC-9 is cationic dye that exhibit potential-dependent accumulation in mitochondria, indicated by a fluorescence emission shift from green (~525 nm) to red (~590 nm). Consequently, mitochondrial depolarization is indicated by a decrease in the red/green fluorescence intensity ratio. The potential-sensitive color shift is due to concentration dependent formation of red fluorescent J-aggregates.

The ratio of green to red fluorescence is dependent only on the membrane potential and not on other factors such as mitochondrial size, shape, and density that may influence single-component fluorescence signals. Use of fluorescence ratio detection therefore allows researchers to make comparative measurements of membrane potential and determine the percentage of mitochondria within a population that respond to an applied stimulus. Subtle heterogeneity in cellular responses can be discerned in this way.

Guidelines for Use

Preparing the Stock Solutions

Stock solutions can be prepared at 1-5 mg/mL in DMSO. A convenient procedure for storing stock solutions is to divide them into portions, each sufficient for one day of experimental work, and store them in a freezer (≤–20°C) until required for use.

Fluorescence Microscopy Staining

Typical staining protocols abstracted from the research literature are summarized in Table 1.

Following incubation in dye-containing medium, it is usual to wash the cells before starting experimental observations.

Optical Filters

A number of different optical filter configurations can be used for analysis of JC-9 by fluorescence microscopy (Table 2). For confocal laser scanning microscopy, the monomer and J-aggregate forms can be excited simultaneously by 488 nm argon-ion laser sources. The J-aggregate form can be excited selectively using the 568 nm argon-krypton laser line.

Appearance

Polarized mitochondria are marked by punctate orange-red fluorescent staining. On depolarization, the orange-red punctate staining is replaced by diffuse green monomer fluorescence. Some of the green fluorescence may remain associated with mitochondria, due to potential-independent interactions of the JC-9 monomer with mitochondrial membranes.

Flow Cytometry Staining

Typical staining protocols abstracted from the research literature are summarized in Table 1. Dissociated cells for flow cytometric analysis are diluted to a density of about 1 × 10^6 cells/mL for staining.

Detector Configuration

When excited simultaneously by 488 nm argon-ion laser sources, the JC-9 monomer and J-aggregate can be detected separately in the conventional flow cytometer FL1 and FL2 channels respectively.

Table 1. JC-9 cell staining conditions.

Cell Type	Incubation Conditions		
	Dye Conc.	Temperature	Time
Neurons (rat)	2.0 µg/mL	37°C	20-30 min
Human fibroblasts	0.3 µg/mL	37°C	1 h
O-2A oligodendrocytes	10 µg/mL	37°C	10 min
PC12	10 µg/mL	37°C	10 min
Colo-205	10 µg/mL	37°C	10 min
U937	10 µg/mL	22°C	10 min
Cardiac myocytes (rat)	10 µg/mL	37°C	10 min

Table 2. Optical filters for fluorescence microscope imaging of JC-9.

Species Detected	Excitation	Dichroic	Emission
Monomer alone	485 ±11 nm	505 nm	530 ±15 nm
J-aggregate alone	535 ± 17.5 nm	570 nm	590 ±17.5 nm
Monomer and J-aggregate, simultaneous	475 ± 20 nm	505 nm	≥510 nm
Monomer and J-aggregate, simultaneous	485 ± 11 nm	505 nm	530 ±15 AND ≥590 nm