JC-1 and JC-9 Mitochondrial Potential Sensors

Table 1. Contents and storage information.

Material	Amount	Storage	Stability
JC-1; $CBIC_2(3)$	5 mg *	• 2–6°C • Desiccate • Protect from light	When stored as directed, product should be stable for 6 months.
JC-9; $DiNOC_1(3)$	5 mg †		

* JC-1 concentrations of 1–5 mg/mL correspond to 1.5–7.7 mM, MW = 652. † JC-9 concentrations of 1–5 mg/mL correspond to 1.9–9.4 mM, MW = 532.

Approximate fluorescence excitation/emission maxima: 514/529 nm, monomer form; 585/590 nm J-aggregate form.

Introduction

JC-1 and JC-9 are cationic dyes (Figure 1) 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.[1–3] JC-1 can be used as an indicator of mitochondrial potential in a variety of cell types, including myocytes [3] and neurons,[4] as well as in intact tissues [5] and isolated mitochondria.[6] JC-1 is more specific for mitochondrial versus plasma membrane potential, and more consistent in its response to depolarization, than other cationic dyes such as $DiOC_6(3)$ and rhodamine 123.[7]

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.[1,6] For example, four distinct patterns of mitochondrial membrane potential change in response to glutamate receptor activation in neurons have been identified using confocal ratio imaging of JC-1 fluorescence.[4] The most widely implemented application of JC-1 is for detection of mitochondrial depolarization occurring in the early stages of apoptosis (Figure 2).[7–10]

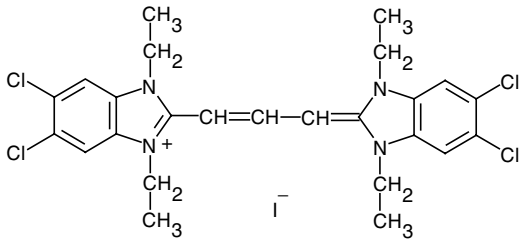

Figure 1. Structure of JC-1 (5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolylcarbocyanine iodide).

Figure 2. Bivariate JC-1 analysis of mitochondrial membrane potential in HL60 cells by flow cytometry. The sensitivity of this technique is demonstrated by the response to K+/valinomycin-induced depolarization (panel B). Distinct populations of cells with different extents of mitochondrial depolarization are detectable following apoptosis-inducing treatment with 5 µM staurosporine for 2 hours (panel C). (Figure kindly supplied by Dr. Andrea Cossarizza, University of Modena and Reggio Emilia.)

Guidelines for Use

Materials Required but Not Provided

- DMSO
- DMF

Preparing the Stock Solutions

Stock solutions can be prepared at 1–5 mg/mL in dimethylsulfoxide (DMSO) or dimethylformamide (DMF). 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.[3]

Fluorescence Microscopy

Staining

Typical staining protocols abstracted from the research literature are summarized in Table 2. 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-1 or JC-9 by fluorescence microscopy (Table 3). 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-1 monomer with mitochondrial membranes.[2,3]

Flow Cytometry

Staining

Typical staining protocols abstracted from the re-search literature are summarized in Table 2. 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-1 monomer and J-aggregate can be detected separately in the conventional flow cytometer FL1 and FL2 channels respectively (Figure 2).

Table 2. JC-1 cell staining conditions.

Cell Type	Adherent or Dissociated	Incubation Conditions			Analysis Method
		Dye Concentration	Temperature	Time	
Neurons (rat) [1]	Adherent	2.0 µg/mL	37°C	20–30 min	Confocal microscope
Neurons (rat) [2]	Adherent	1.0 µg/mL	37°C	20 min	Confocal microscope
Human fibroblasts [3]	Dissociated	0.3 µg/mL	37°C	1 hour	Flow cytometer
O-2A oligodendrocytes (rat) [4]	Adherent	10 µg/mL	37°C	10 min	Wide-field microscope
PC12 [5]	Adherent	10 µg/mL	37°C	10 min	Confocal microscope
Colo-205 [6]	Dissociated	10 µg/mL	37°C	10 min	Flow cytometer
U937 [7]	Dissociated	10 µg/mL	22°C	10 min	Flow cytometer
Cardiac myocytes (rat) [8]	Dissociated	10 µg/mL	37°C	10 min	Wide-field microscope

1. J Neurosci 16, 5688 (1996); **2.** Neuron 15, 961 (1995); **3.** Exp Cell Res 245, 170 (1998); **4.** J Physiol 508, 413 (1998); **5.** Neuronal precursor cell line, J Neurosci 18, 932 (1998); **6.** Human colon adenocarcinoma, J Cell Biol 138, 449 (1997); **7.** Human premonocytic cell line, Proc Natl Acad Sci USA 93, 6458 (1996), Biochem Biophys Res Comm 197, 40 (1993); **8.** J Physiol 486, 1 (1995).

Table 3. Optical filters for fluorescence microscope imaging of JC-1.

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

References

1. Proc Natl Acad Sci U S A 88, 3671 (1991); **2.** Biochemistry 30, 4480 (1991); **3.** J Physiol 486, 1 (1995); **4.** J Neurosci 16, 5688 (1996); **5.** Methods 18, 104 (1999); **6.** Exp Cell Res 222, 84 (1996); **7.** FEBS Lett 411, 77 (1997); **8.** J Neurosci 18, 932 (1998); **9.** J Cell Biol 138, 449 (1997); **10.** Exp Cell Res 245, 170 (1998).

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

Cat #	Product Name	Unit Size
D22421	3,3′-dimethyl-α-naphthoxacarbocyan ine iodide (JC-9; $DiNOC_1(3)$)	5 mg
T3168	5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolylcarbocyanine iodide (JC-1; $CBIC_2(3)$)	5 mg

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