

## JC-1 and JC-9 Mitochondrial Potential Sensors

### Quick Facts

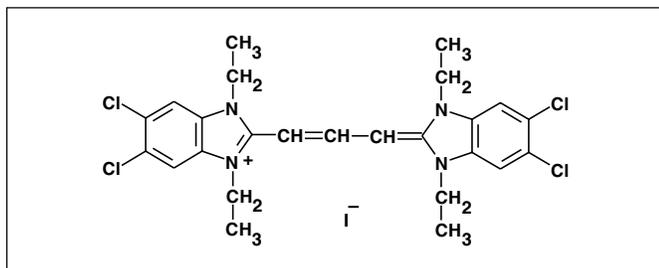
#### Storage upon receipt:

- 4°C
- Desiccate
- Protect from light

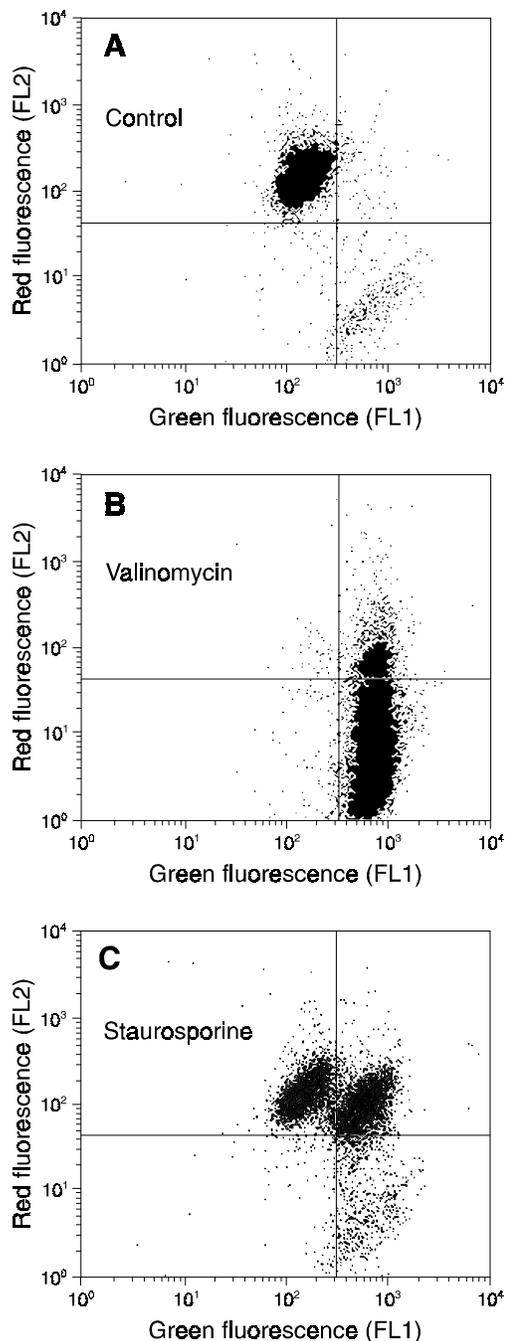
### Introduction

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.<sup>1-3</sup> JC-1 can be used as an indicator of mitochondrial potential in a variety of cell types, including myocytes<sup>3</sup> and neurons,<sup>4</sup> as well as in intact tissues<sup>5</sup> and isolated mitochondria.<sup>6</sup> 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<sub>6</sub>(3) and rhodamine 123.<sup>7</sup>

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.<sup>1,6</sup> 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.<sup>4</sup> The most widely implemented application of JC-1 is for detection of mitochondrial depolarization occurring in the early stages of apoptosis (Figure 2).<sup>7-10</sup>



**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<sup>+</sup>/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)

**Table 1.** JC-1 cell staining conditions.

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

### Storage and Handling

JC-1 and JC-9 are supplied in solid form in units of 5 mg. The solid dyes can be stored in a light-shielded, desiccated container at room temperature. Stock solutions can be prepared at 1–5 mg/mL in dimethylsulfoxide (DMSO) or dimethylformamide (DMF).

JC-1 concentrations of 1–5 mg/mL correspond to 1.5–7.7 mM (the molecular weight is 652). For JC-9 (molecular weight = 532), 1–5 mg/mL corresponds to 1.9–9.4 mM. 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.<sup>3</sup>

### Applications

#### 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-1 or JC-9 by fluorescence

microscopy. 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.<sup>2,3</sup>

#### 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  $1 \times 10^6$  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.** Optical filters for fluorescence microscope imaging of JC-1 and 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

### 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 Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
D-22421	3,3'-dimethyl- $\alpha$ -naphthoxacarbocyanine iodide (JC-9; DiNOC <sub>1</sub> (3)) .....	5 mg
T-3168	5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolylcarbocyanine iodide (JC-1; CBIC <sub>2</sub> (3)) .....	5 mg

---

**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 Leiden, the Netherlands. All others should contact our Technical Assistance Department in Eugene, Oregon.

Please visit our Web site — [www.probes.com](http://www.probes.com) — for the most up-to-date information

**Molecular Probes, Inc.**

29851 Willow Creek Road, Eugene, OR 97402-0469  
Phone: (541) 465-8300 • Fax: (541) 344-6504

**Customer Service:** 7:00 am to 5:00 pm (Pacific Time)  
Phone: (541) 465-8338 • Fax: (541) 344-6504 • [order@probes.com](mailto:order@probes.com)

**Toll-Free Ordering for USA and Canada:**  
Order Phone: (800) 438-2209 • Order Fax: (800) 438-0228

**Technical Assistance:** 8:00 am to 4:00 pm (Pacific Time)  
Phone: (541) 465-8353 • Fax: (541) 465-4593 • [tech@probes.com](mailto:tech@probes.com)

**Molecular Probes Europe BV**

PoortGebouw, Rijnsburgerweg 10  
2333 AA Leiden, The Netherlands  
Phone: +31-71-5233378 • Fax: +31-71-5233419

**Customer Service:** 9:00 to 16:30 (Central European Time)  
Phone: +31-71-5236850 • Fax: +31-71-5233419  
[eurorder@probes.nl](mailto:eurorder@probes.nl)

**Technical Assistance:** 9:00 to 16:30 (Central European Time)  
Phone: +31-71-5233431 • Fax: +31-71-5241883  
[eurotech@probes.nl](mailto:eurotech@probes.nl)

*Molecular Probes' products are high-quality reagents and materials intended for research purposes only. These products must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Please read the Material Safety Data Sheet provided for each product; other regulatory considerations may apply.*

Several of Molecular Probes' products and product applications are covered by U.S. and foreign patents and patents pending. Our products are not available for resale or other commercial uses without a specific agreement from Molecular Probes, Inc. We welcome inquiries about licensing the use of our dyes, trademarks or technologies. Please submit inquiries by e-mail to [busdev@probes.com](mailto:busdev@probes.com). All names containing the designation ® are registered with the U.S. Patent and Trademark Office.

Copyright 2002, Molecular Probes, Inc. All rights reserved. This information is subject to change without notice.