BIOLUMINOR

JC-1 for Labeling of Mitocondria

Table 1 Contents and storage

Material	Amount	Storage	Stability
JC-1	1mg 5mg	♦ 2-6℃ ♦ Desiccate ♦ Protect from light	When stored as directed, products are stable for at least 6 months
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 luorescence excitation/emission maxima: 514/529 nm, monomer form; 585/590 nm J-aggregate form.			

The membrane potential of energized mitochondria (negative inside) can promoted a directional uptake of JC-1 into the matrix, with subsequent formation of J-aggregates. And the potential-sensitive color shift is due to formation of red fluorescent J-aggregates. Consequently, the polarization of mitochondrial membrane will decrease the green/ red fluorescence intensity ratio, shown as the fluorescence emission shift from green (~527 nm) to red (~590 nm) when excited at 488nm.

JC-1 can be used as an indicator of mitochondrial potential in a variety of cell types, including myocytes and neurons, as well as in intact tissues and isolated mitochondria. 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 DiOC6(3) and rhodamine 123.

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.

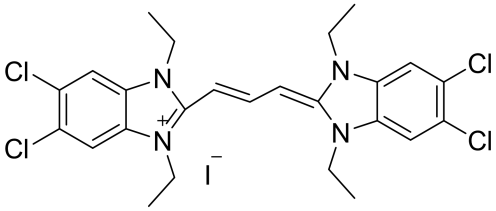

Fig. 1 Structure of JC-1.

Photophysical properties of JC-1

Fig. 2 shows that the optimal fluorescence excitation of JC-1 is at 488 nm while the maximal fluorescence emission intensity is at 527nm (green, monomer) and 590 nm (red, aggregate).

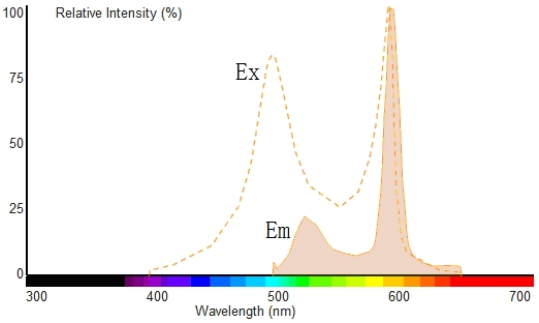

Fig. 2 Fluorescence Ex/Em (excited at 488 nm) spectra of JC-1 in pH 8.2 buffer containing 0.01 (v/v) DMSO.

Materials Required but Not Provided

DMSO
DMF

Preparing 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.

Fluorescence Microscopy

Cell culture

1. Culture cells in multi-well plates or tissue culture flasks to a density optimal for apoptosis induction (typically ≤ $1x10^6$ cells/mL) according to your protocol. Duplicate or triplicate wells/flasks should be prepared for each experimental group.

CAUTION: Cells cultivated at densities $>10^6$/mL may begin to naturally enter apoptosis. The cell concentration and protocol for inducing apoptosis should be optimized for each experimental system.

2. Be sure to include at least one well or flask of untreated cells for each cell type and treatment condition to serve as control cells.
3. Induce apoptosis according to your protocol.
4. During the last 5 minutes of apoptosis induction, prepare the FL1-only positive fluorescence control sample by adding 1 μL of 50 mM CCCP per 1 mL culture volume to the untreated cell samples (Cf = 50 μM).

Preparation of JC-1 Staining Solution

(Begin preparation 30 minutes prior to the end of cell treatment.)

1. Warm fresh culture medium to 37° C.
2. Thaw an aliquot of the JC-1 Reagent at room temperature (RT). Make sure JC-1 is completely thawed and warmed to RT before diluting.
3. Mix thawed JC-1 well before dilution.
4. Prepare the JC-1 Staining Solution by diluting the reagent 1:10 in pre-warmed culture media.
5. Mix well to dissolve all particulates.

CAUTION: Do not centrifuge the JC-1 reagent.

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.

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Optical Filters: A number of different optical filter configurations can be used for analysis of JC-1 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 FL1 and FL2 channels respectively.

associated with mitochondria, due to potential independent interactions of the JC-1 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 × 106 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

Table.1 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.2 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. *Biochemistry*, 1991, 30, 4480
2. *FEBS Lett.*, 1997, 411, 77
3. *J. Physiol.*, 1995, 486, 1
4. *Exp. Cell Res.*, 1996, 222, 84