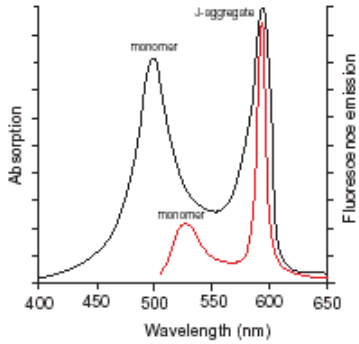
JC-1

An useful probe for mitochondria potential studies, notably for cell viability and apoptosis

Product Information

Name :	**JC-1; CBIC2(3), iodide salt** 5,5',6,6'-tetrachloro-1,1',3,3'- tetraethylbenzimidazolylcarbocyanine iodide
Catalog Number :	FP-52314A, 5mg
Structure :	$C_{25}H_{27}Cl_4IN_4$
Molecular Weight :	MW= 652.2
Solubility in:	DMSO, DMF
Absorption / Emission :	$\lambda_{exc}\backslash\lambda_{em}$ (monomer) = 490 / 527 nm $\lambda_{exc}\backslash\lambda_{em}$ (J-aggregate) = 585(*) / 595 nm (*) vary according experimental conditions
EC (M^{-1} cm^{-1}) **:**	190 000

Name :	**JC-1 , chloride salt** 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethyl-benzimidazolylcarbocyanine chloride
Catalog Number :	FP-AM304A, 5 mg
Structure :	$C_{25}H_{27}Cl_3N_4$
Molecular Weight :	MW= 561.5
Solubility in:	DMSO
Absorption / Emission :	$\lambda_{exc}\backslash\lambda_{em}$ (monomer) = 490 / 527 nm $\lambda_{exc}\backslash\lambda_{em}$ (J-aggregate) = 585(*) / 595 nm (*) vary according experimental conditions
EC (M^{-1} cm^{-1}) **:**	180 000



Absorption and fluorescence emission (excited at 488nm) spectra of JC-1 in pH 8.2 buffer containing 1 % (v/v) DMSO.

Storage: +4°C (or –20°C for long term with aliquotes)(M) Protect from light and moisture

Introduction

JC-1 is a fluorescent cationic dye and 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 by a fluorescence emission shift from green (~527nm) to red (~595nm).
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.

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.
In non-apoptotic cells, JC-1 aggregates in the mitochondria (J-aggregates) resulting in **red** fluorescence (Abs/Em = 585/595). The brightness of red fluorescence is proportional to mitochondrial membrane potential and varies among different types. In apoptotic and necrotic cells, JC-1 exists in monomeric form and stains cells **green** .
Therefore, JC-1 permits to distinct non-apoptotic red fluorescent cells and apoptotic green fluorescent cells. A mitochondrial depolarization is indicated by a decrease in the red/green fluorescence intensity ratio.

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. The percentage of mitochondria within a population that respond to an applied stimulus can thus be determined . JC-1 is notably for detection of mitochondrial depolarization occurring in the early stages of apoptosis.

Two forms of JC-1 are avalaible. They have identical spectral properties. The iodide form has been used in most of the publication. However, some researchers may prefer the chloride form since Cl^- is the most prevalent anion in biological systems

Directions for use

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Handling and Storage

Stock solutions can be prepared at 1-5 mg/mL in dimethylsulfoxide (DMSO) or dimethylformamide (DMF) or MeOH, aliquote in one day quantities and frozen.

The filter should be choosen according following figures:

Species Detected	Excitation	Dichroic	Emission
Monomer alone	490 nm	505 nm	527 ± 15 nm
J-aggregate alone	535 ± 17.5 nm 570 nm		595 ± 17.5 nm

Polarized mitochondria show, upon JC-1 staining, orange-red fluorescent points.
On depolarization, the orangered punctate staining is replaced by diffuse green monomer fluorescence.
Note: Some of the green fluorescence may remain associated with mitochondria, due to potential-independent interactions of the JC-1 monomer with mitochondrial membranes.

Guidelines for use – in Flow Cytometer [0]

1- Total cell count should be between $5x10^5$ and $1x10^6$ cells/ml. Dye concentrations and incubation time may vary depending on cell type and will need to be determined independently.

2- Add 1 µl of JC-1 at a 10 mM stock for a final concentration of 10 µM.

3- The cells are incubated at +37°C for 30 minutes to load the dye. No PI is added to this sample. It has been found that adding the cells to the dye already in a tube allows for better loading of this dye.

4- Cells are examined by exciting the dyes/PI with a 488 nm laser. A standard optical set-up is used on Flow cytometer. JC-1 monomers and aggregates are detected in FL-1 and FL-2, respectively.

Other protocol may found in the literature [(m)] [(n)]

Guidelines for use – in Fluorescent Microscopy [0]

Staining of Cells in Suspension

1- Incubate 1 x 10^6 cells/ml in 0.5 ml JC-1 reagent at +37°C in a 5% CO2 incubator or at room temperature for 15 minutes or less.
Note: Do not incubate cells in JC-1 working solution for more than 15 min since JC-1 precipitants may appear.

2- Centrifuge for 5 min at 400 x g and remove supernatant. Resuspend the cell pellet in 2 ml buffer followed by centrifugation. Remove supernatant. Resuspend the cell pellet in 0.3 ml Assay Buffer.

3- Observe **immediately** with a fluorescence microscope using a "dual-bandpass" filter designed to simultaneously detect fluorescein and rhodamine or fluorescein.
In live non-apoptotic cells, the mitochondria will appear red following aggregation of the JC-1 reagent. The red aggregates emit at 590 nm. In apoptotic and dead cells, the dye will remain in its monomeric form and will appear green with an emission at 530 nm

Staining of Monolayer Cells

1- Grow cells on a glass cover slip in a petri dish or in a chamberslide. Induce cells according to your specific protocol.

2- Remove the cell culture media and incubate with diluted JC-1 reagent sufficient to cover the cells, at +37°C in a 5% CO2 incubator or at room temperature for 15 minutes or less.
Note: Do not incubate cells in JC-1 working solution for more than 15 min since JC-1 precipitants may appear.

3- Remove media and wash once with Buffer. Add a drop of PBS and cover with a coverslip.

4- Observe **immediately** with a fluorescence microscope using a "dual-bandpass" filter designed to simultaneously detect fluorescein and rhodamine or fluorescein.
In live non-apoptotic cells, the mitochondria will appear red following aggregation of the JC-1 reagent. The red aggregates emit at 590 nm. In apoptotic and dead cells the dye will remain in its monomeric form and will appear green with an emission at 530 nm.

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Related products

- JC-1 Kit (with assay buffer), FP-52314B
- JC-10, superior alternative to JC-1, CL0440
- JC-9 (DINOC(3), R1220A
- Valinomycin, FP-09246B
- CCCP, 091640
- DMSO, anhydrous, FP-JW7390
- TMRM, FP-21089A
- Dihydrorhodamine 123, FP-AM352A
- Nonyl acridine orange, FP-58566A (long term studies)

Ordering information

Catalog size quantities and prices may be found at http://www.fluoprobes.com
Please inquire for higher quantities (avaibility, shipment conditions).

For any information, please ask : Fluoprobes; Hotline : +33(0)4 70 03 73 06

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