JC-1 (Iodide)

Dyes and Stains

Fluorescent lipophilic dye used to quantify mitochondrial membrane potential and assess cell viability

Catalog #100-0993

5 mg

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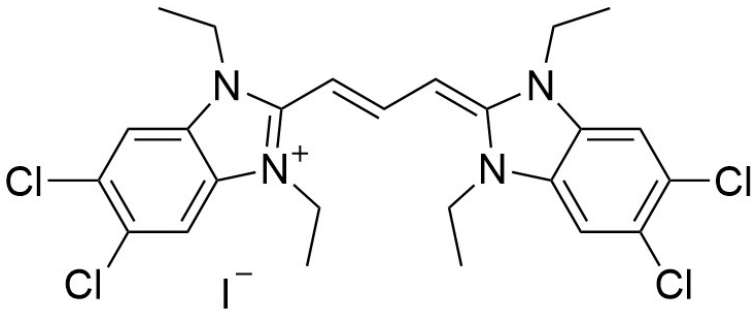
Product Description

JC-1 (Iodide) is a lipophilic, cationic carbocyanine dye which can visualize high and low mitochondrial membrane potentials used in flow cytometry and fluorescence microscopy applications. This dye can reversibly change its fluorescence, where it is in equilibrium between its monomeric and aggregate form. JC-1 exists as a monomer in polarized mitochondria, and upon excitation emits a green fluorescence with an emission maximum of 530 nm. J-aggregates form at higher concentrations, emitting a green-orange fluorescence with an emission maximum of 590 nm. The maintenance of JC-1 aggregates is dependent on the electrochemical gradient. JC-1 can be used both qualitatively to measure a shift in fluorescence emission, or quantitatively to detect the difference in fluorescence intensity in channels FL1 (FITC) and FL2 (PE). The green-to-orange fluorescence intensity ratio is used to evaluate mitochondrial depolarization occurring in apoptosis (Sivandzade et al.).

Alternative Names:	5,5',6,6'-Tetrachloro-1,1',3,3'-tetraethylbenzimidazolylcarbocyanine iodide; 5,5′,6,6′-Tetrachloro-1,1′,3,3′-tetraethyl-imidacarbocyanine iodide; CBIC2(3); JC-1
CAS Number:	3520-43-2
Chemical Formula:	$C_{25}H_{27}Cl_4IN_4$
Molecular Weight:	652.23 g/mol
Chemical Name:	5,6-Dichloro-2-[(*E*)-3-(5,6-dichloro-1,3-diethyl-1,3-dihydro-2*H*-benzimidazol-2-ylidene)-1-prop-1-enyl]-1,3-diethyl-1*H*-benzimidazolium iodide
Excitation Wavelength:	515 nm
Emission Wavelength:	530 nm
Extinction Coefficient:	195,000 $cm^{-1}M^{-1}$
Structure:	



Properties

Storage:	Store at -20°C.
Shelf Life:	Stable until expiry date (EXP) on label. Protect product from prolonged exposure to light.
Format:	Red powder

Directions for Use

Please read the entire protocol before proceeding. The following protocol is for staining cells in a 96- or 384-well plate. If using other cultureware, adjust volumes accordingly. Treat cells as desired before preparing the JC-1 (Iodide) working solution.

Preparation of JC-1 (Iodide) Stock and Working Solutions

1. To prepare a stock solution, dissolve JC-1 (Iodide) in dimethyl sulfoxide (DMSO) at 2 - 10 mM. Mix thoroughly.

   NOTE: If not used immediately, aliquot and store at -20°C. Do not exceed the expiry date as indicated on the label. After thawing aliquots, use immediately; do not re-freeze.
2. To prepare a JC-1 (Iodide) working solution, dilute the stock solution (prepared in step 1) to 10 - 30 µM with Hanks' Balanced Salt Solution with 20 mM HEPES (HHBS) or buffer of your choice. Vortex to mix well. Use the working solution immediately; do not store.

   NOTE: To increase the solubility of JC-1 (Iodide), add 0.04% Pluronic® F-127 (AAT Bioquest Catalog #20053).

   NOTE: To avoid aggregation of JC-1 (Iodide) in solution, filter the working solution with a 0.4 µm filter immediately before use.

Staining Samples

Refer to section A for use with a fluorescence microplate reader or section B for use with a fluorescence microscope or flow cytometer.

A. FLUORESCENCE MICROPLATE READER

1. To induce apoptosis, treat cells with test compounds and incubate at 37°C and 5% CO_2 for an appropriate length of time. Prepare untreated cells as a control.

   Example: Jurkat cells treated with staurosporine require ~2 hours of incubation for apoptosis to occur.

   NOTE: The optimal cell density to induce apoptosis should be determined for different cell lines.
2. To prepare blank wells, add the same volume of compound and buffer into culture medium without cells.
3. Add the JC-1 (Iodide) working solution to the plate as follows:
   - 96-well plate: 100 µL/well
   - 384-well plate: 25 µL/well
4. Incubate the plate at 37°C and 5% CO_2 for 15 - 60 minutes.

   NOTE: The optimal incubation time should be determined for different cell lines.
5. Remove the JC-1 (Iodide) working solution and wash cells with HHBS or a buffer of choice.
6. Add HBBS to the plate as follows:
   - 96-well plate: 100 µL/well
   - 384-well plate: 25 µL/well
7. Monitor the fluorescence intensity using a fluorescence microplate reader at Ex/Em = 490/525 nm and 490/590 for ratio analysis.

A. FLUORESCENCE MICROSCOPE OR FLOW CYTOMETER

1. Plate 1 - 5 x 10^5 cells per well of a 96-well plate.
2. To induce apoptosis, treat cells with test compounds and incubate at 37°C and 5% CO_2 for an appropriate length of time. Prepare untreated cells as a control.

   Example: Jurkat cells treated with staurosporine require ~2 hours of incubation for apoptosis to occur.

   NOTE: The optimal cell density to induce apoptosis should be determined for different cell lines.
3. Centrifuge the cells at 300 - 500 x *g* for 5 minutes.
4. Remove supernatant and add 100 µL/well of the JC-1 (Iodide) working solution to the cells.
5. Incubate at room temperature (15 - 25°C) or 37°C for 10 - 30 minutes; protect from light.
6. Centrifuge the cells at 300 - 500 x *g* for 5 minutes.
7. Remove supernatant and wash cells twice with HHBS. Resuspend cells in 100 - 200 µL of HHBS.
8. Monitor the fluorescence intensity using a fluorescence microscope or flow cytometer. Refer to Table 1 for recommended instrument configurations.

Table 1. Recommended Instrument Configurations for Fluorescence Detection

	FLUORESCENCE MICROSCOPE	FLOW CYTOMETER
Excitation	490 nm	488 nm laser
Emission	525 nm (590 nm for ratio analysis)	530/30 nm, 575/26 nm filter

References

1. Chaoui D et al. (2006) JC-1, a sensitive probe for a simultaneous detection of P-glycoprotein activity and apoptosis in leukemic cells. Cytom Part B - Clin Cytom 70(3): 189–96.
2. Sivandzade F et al. (2019) Analysis of the mitochondrial membrane potential using the cationic JC-1 dye as a sensitive fluorescent probe. Bio-Protocol 9(1): 1–13.