

Product Data Sheet

Product Name: JC-1
Cat. No.: GC12331

Chemical Properties

Cas. No. 3520-43-2

Chemical Name 5,6-dichloro-2-[(E)-3-(5,6-dichloro-1,3-diethylbenzimidazol-3-ium-2-yl)prop-2-enylidene]-1,3-diethylbenzimidazole;iodide

SMILES CCN1C2=CC(=C(C=C2[N+](=C1C=CC=C3N(C4=CC(=C(C=C4N3CC)Cl)Cl)CC)CC)Cl)Cl.[I-]

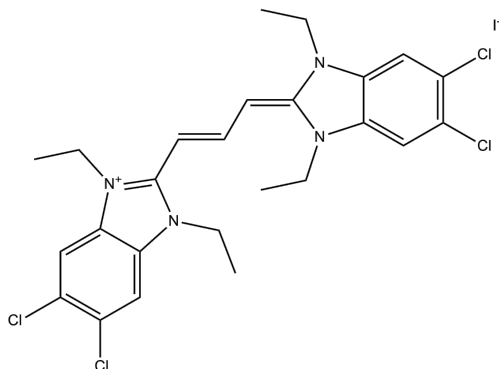
Formula $C_{25}H_{27}Cl_4IN_4$ M.Wt 652.23

Solubility ≥ 32.6 mg/mL in DMSO with gentle warming Storage Store at $-20^{\circ}C$, protect from light

General tips For obtaining a higher solubility, please warm the tube at $37^{\circ}C$ and shake it in the ultrasonic bath for a while. Stock solution can be stored below $-20^{\circ}C$ for several months.

Shipping Condition Evaluation sample solution: ship with blue ice All other available size: ship with RT, or blue ice upon request.

Structure



Protocol

Protocol for mitochondrial membrane potential cell assay [1]:

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1. Prepare a 5mg/mL stock solution of JC-1 in DMSO (~7.7 mM). JC-1 is light sensitive. Do not expose to direct intense light. Perform all staining procedures in subdued light. Avoid repeated freeze/thawing of the JC-1 stock solution. Make small aliquots after the first thaw and store them at -20°C.
2. Grow cells on a glass cover slip in a petri dish or in a chamber slide. Induce the cells according to your specific protocol.
3. Warm fresh culture medium to 37°C. Thaw an aliquot of the JC-1 stock solution at room temperature (RT). Make sure JC-1 is completely thawed and warmed to RT before diluting. Mix thawed JC-1 well before dilution. Prepare the JC-1 staining solution by diluting the reagent in pre-warmed culture media. For the staining of adherent cells, JC-1 is diluted in medium to a final concentration of ~ 5 ug/mL (vortex during dilution to prevent the formation of precipitates). Mix well to dissolve all particulates. Do not centrifuge the reagent. Dilute JC-1 reagent immediately prior to use.
4. Remove the cell culture media and replace with enough diluted 1X JC-1 reagent sufficient to cover the cells.
5. Incubate the cells for 10 min at 37°C (or RT for 15 min). The duration of the staining depends upon the cell type.
6. Remove the media and wash the monolayer twice with PBS or fresh media.
7. Add a drop of PBS and cover the cells with a cover slip.
8. Observe cells immediately with a fluorescence microscope using a dual-band pass filter designed to simultaneously detect fluorescein and rhodamine or fluorescein and Texas Red. In live non-apoptotic cells, the mitochondria will appear red following aggregation of the JC-1 reagent. The red aggregates emit at about 590 nm. In apoptotic and dead cells the dye will remain in its monomeric form and will appear green with an emission at about 530 nm.

Protocol for functional assay JC-1 for P-glycoprotein (Pgp) [2]:

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1. 1.For staining, cells were washed twice and resuspended in PBS containing 0.1 μ M JC-1 monomer at a concentration of 5×10^5 cells/mL and incubated at 37°C for 15 without or with modulator to assess Pgp function.
2. 2.Cells were washed twice in cold PBS, and samples were analyzed.
3. 3.Cell fluorescence was recorded using a FACSort flow cytometer equipped with a 488-nm argon laser and 3 fluorescence detectors: FL1 (530-nm band-pass filter), FL2 (585-nm band-pass filter), and FL3 (650 nm band-pass filter).
4. 4.JC-1 fluorescences were analyzed on the FL1 and FL2 channels for detection of the fluorescence of the dye monomer and liquid crystal form, respectively. The function of Pgp was established with blast cells selected by CD34 antibody or with physical characteristics only if blast cells did not express characteristic markers.

This protocol only provides a guideline, and should be modified according to your specific needs.

References:

- [1]. Perelman A, Wachtel C, et.al. JC-1: alternative excitation wavelengths facilitate mitochondrial membrane potential cytometry. Cell Death Dis. 2012 Nov 22;3(11):e430. doi: 10.1038/cddis.2012.171. PMID: 23171850; PMCID: PMC3542606.
- [2]. Legrand O, Perrot JY, et.al. JC-1: a very sensitive fluorescent probe to test Pgp activity in adult acute myeloid leukemia. Blood. 2001 Jan 15;97(2):502-8. doi: 10.1182/blood.v97.2.502. PMID: 11154229.

Background

JC-1(CBIC2) is a cationic carbonyl cyanine fluorescent dye.JC-1 serves as an excellent fluorescent probe widely employed for the detection of mitochondrial membrane potential. It selectively enters the mitochondria and changes its fluorescence properties with changes in mitochondrial transmembrane potential ($\Delta\psi$ m) allowing for the assessment of membrane potential in cells, tissues, or purified mitochondria. In healthy mitochondria, JC-1 forms aggregates within the mitochondrial matrix, giving rise to intense red fluorescence (Ex=585 nm, Em=590 nm).Conversely, under conditions of low mitochondrial membrane potential, JC-1 fails to aggregate in the matrix, resulting in the emission of

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green fluorescence (Ex=514 nm, Em=529 nm) [1-4].

JC-1, a fluorescent molecule, is also a probe of P-glycoprotein (Pgp). A specific property of JC-1, due to its stacking in a liquid crystal form, is the dependence of its fluorescence emission wavelength on its concentration. On excitation at 490 nm, JC-1 monomers display a cytoplasmic fluorescence emission centered at 537 nm (green band). Beyond a critical concentration, JC-1 aggregates in mitochondrion, thus leading to the emergence of an intense emission band centered at 597 nm (red band), in addition to the cytoplasmic green band. We have shown in cell lines that sensitive cells display both green and red fluorescence. In resistant cells, when Pgp activity increased, green fluorescence of JC-1 decreased and red fluorescence was lost. In intermediate-resistant cells, green fluorescence intensity was often identical to the fluorescence intensity of sensitive cells, but red fluorescence was already lost [5-7].

References:

- [1]. Perelman A, Wachtel C, et.al. JC-1: alternative excitation wavelengths facilitate mitochondrial membrane potential cytometry. *Cell Death Dis.* 2012 Nov 22;3(11):e430. doi: 10.1038/cddis.2012.171. PMID: 23171850; PMCID: PMC3542606.
- [2]. Chazotte B. Labeling mitochondria with JC-1. *Cold Spring Harb Protoc.* 2011 Sep 1;2011(9):pdb.prot065490. doi: 10.1101/pdb.prot065490. PMID: 21880824.
- [3]. Armon-Omer A, Neuman H, et.al. Mitochondrial activity is impaired in lymphocytes of MS patients in correlation with disease severity. *Mult Scler Relat Disord.* 2020 Jun;41:102025. doi: 10.1016/j.msard.2020.102025. Epub 2020 Feb 25. PMID: 32146432.
- [4]. Gravance CG, Garner DL, et.al. Assessment of equine sperm mitochondrial function using JC-1. *Theriogenology.* 2000 Jun;53(9):1691-703. doi: 10.1016/s0093-691x(00)00308-3. PMID: 10968415.
- [5]. Legrand O, Perrot JY, et.al. JC-1: a very sensitive fluorescent probe to test Pgp activity in adult acute myeloid leukemia. *Blood.* 2001 Jan 15;97(2):502-8. doi: 10.1182/blood.v97.2.502. PMID: 11154229.
- [6]. Kühnel JM, Perrot JY, et.al. Functional assay of multidrug resistant cells using JC-1, a carbocyanine fluorescent probe. *Leukemia.* 1997 Jul;11(7):1147-55. doi: 10.1038/sj.leu.2400698. PMID: 9205004.

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[7].Smiley ST, Reers M, et.al. Intracellular heterogeneity in mitochondrial membrane potentials revealed by a J-aggregate-forming lipophilic cation JC-1. Proc Natl Acad Sci U S A. 1991 May 1;88(9):3671-5. doi: 10.1073/pnas.88.9.3671. PMID: 2023917; PMCID: PMC51514.

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