Revised: December 14, 2022

Product Information

Aquaphile™ JC-1, 1.5 mM in DMSO

Catalog Number: 70076

Unit Size: 50 uL

Storage and Handling

Store at 4°C, protected from light. Product is stable for at least 1 year from date of receipt when stored as recommended.

Spectral Properties:

Ex/Em (green monomeric dye): 510/527 nm
Ex/Em (red aggregated dye): 585/590 nm

Product Description

JC-1 is a widely used mitochondrial dye that stains mitochondria in living cells in a membrane potential-dependent fashion. JC-1 monomer is in equilibrium with so-called J-aggregates, which are favored at higher dye concentrations or higher mitochondrial membrane potential. Monomeric JC-1 emits green fluorescence at 527 nm, while the J-aggregates emit red fluorescence at 590 nm. Therefore, it is possible to quantify mitochondrial potential changes with fluorescence ratioing. In healthy cells, the dye localizes to mitochondria with red fluorescence, while in apoptotic cells with depolarized mitochondrial potential, the dye localizes in the cytoplasm and fluoresces green, making JC-1 useful for apoptosis studies. This dye has also been applied in high-throughput drug screening applications.

The tendency of JC-1 to aggregate makes it useful for mitochondrial potential detection but hinders its use in cellular imaging. Because of its hydrophobic nature, JC-1 is prone to aggregate when diluted in buffer and cell culture medium, which can interfere with microscopic imaging and generates a false positive J-aggregate signal outside of cells. To address this issue, Biotium chemists developed a novel Aquaphile™ formulation for JC-1. Unlike alternative dyes like JC-9 and JC-10, Aquaphile™ JC-1 has improved solubility without alterations to the original JC-1 compound chemical structure. The optimized formulation facilitates preparation of staining solutions with minimal dye aggregation compared to JC-1 iodide and JC-1 chloride salts, particularly when staining solution is prepared in cell culture medium. The Aquaphile™ JC-1 stock solution is supplied in DMSO and contains 1.5 mM JC-1 dye.

References

1. Cell Death Disease 3: e430 (2012). doi:10.1038/cddis.2012.171; 2. J Biomol Screen 6(6): 413-20 (2001). doi: 10.1177/108705710100600607; 3. J Neurosci Methods 92(1-2):153-9 (1999). doi: 10.1016/s0165-0270(99)00107-7.

Considerations for staining

- Staining with potential-sensitive mitochondrial dyes must be performed in live cells. Staining does not withstand fixation.
- For optimizing assay conditions, include an uninduced healthy cell control and cells induced to undergo mitochondrial depolarization. For the induced control, we recommend using a mitochondrial decoupler such as carbonyl cyanide chlorophenylhydrazone (CCCP) or a well-characterized apoptosis induction treatment for your cell line to depolarize mitochondrial membrane potential. Each cell line should be evaluated on an individual basis to determine optimal cell density and treatment time for apoptosis induction.
- Staining solution may be prepared in buffer or complete medium with serum. When staining solution is prepared as recommended below, dye aggregation is minimal in buffer but more likely to occur in medium.
- Prepare staining solution immediately before use in buffer or medium pre-warmed to 37°C.
- For staining cells in buffer, PBS or another physiological buffer may be used. For adherent cells, we recommend using a buffer such as HBSS with calcium and magnesium to maintain cell attachment and morphology.
- We recommend testing Aquaphile™ JC-1 at concentrations between 1.5 uM and 5 uM as a starting point to find the optimal concentration for your cell type and application. To avoid cytotoxic effects, use the lowest concentration that gives good results in your assay and keep the final concentration of DMSO below 1%. For a final staining concentration of 3 uM, the 1.5 mM stock solution (which is a 500X concentrate) can be used for 125 assays with 200 uL assay volume. You may scale volumes as needed for your culture vessel.
- JC-1 may be toxic to cells with staining times longer than a few hours and is not recommended for long-term live cell imaging.
- For staining suspension cells for microscopy, use the staining protocol for flow cytometry, then resuspend the cells in 0.3 mL buffer or medium. Mount cells under a coverslip in a drop of buffer or medium. Alternatively, you may transfer cells to a suitable vessel for imaging by microscopy.
- For flow cytometry, 488 nm excitation can be used to detect monomeric and J-aggregate fluorescence from JC-1; however, using 561 nm excitation for J-aggregate detection is more optimal for reducing fluorescence crosstalk from green monomers. It has been reported that using 405 nm excitation for red J-aggregate detection can also effectively control crosstalk from green monomers; however, 405 nm excitation is less efficient for exciting the dye and will produce dimmer signal than 488 nm or 561 nm excitation (see Reference 1).

Experimental Protocols

Preparation of Staining Solution

The useful application of JC-1 relies on its tendency to form dye aggregates. Therefore, even though Aquaphile™ JC-1 is formulated to minimize dye aggregation in buffer, care must still be taken when preparing staining solutions to avoid dye precipitation.

Prepare the staining solution immediately before use. Pipette Aquaphile™ JC-1 stock into an empty tube, and then add prewarmed buffer or medium to bring the dye to the final desired concentration for staining. For example, to prepare 3 uM staining solution, add 1 uL of dye to an empty microcentrifuge tube, then add 500 uL of prewarmed buffer or medium to the tube containing dye and immediately vortex to mix.

Notes:

a. We have found this method produces the most even dye dispersion in aqueous solution. Pipetting the dye directly into the buffer or medium is more likely to generate aggregates.

b. Do not perform serial dilution (e.g., making an intermediate dilution, then diluting again to the final concentration) as this can cause dye aggregation.

Staining Protocol for Fluorescence Microscopy

1. Perform cell treatments. Include an uninduced healthy cell control and cells induced to undergo mitochondrial depolarization.
2. Remove the culture medium from your cells and replace it with the Aquaphile™ JC-1 staining solution.
3. Incubate cells at 37°C for 15-30 minutes.
4. Rinse cells once with fresh prewarmed medium or buffer and add fresh medium or buffer for imaging.

   Note: Rinsing is recommended to remove any extracellular dye aggregates that may have formed but is not required to image JC-1 signal in cells.

5. Observe cells immediately by fluorescence microscopy. In cells with intact mitochondrial membrane potential, the mitochondria will fluoresce red with emission at 590 nm, and the cytoplasm will fluoresce green with emission at 530 nm. In apoptotic and dead cells, the dye will remain in its monomeric form and will appear green. Red JC-1 aggregates can be imaged using optical settings for Cy®3, rhodamine, Texas Red®, or similar fluorophores. The green JC-1 monomers can be imaged using settings for GFP or fluorescein/FITC. Aggregates and monomers can be imaged simultaneously with a dual band-pass filter for fluorescein/rhodamine.

Staining Protocol for Flow Cytometry

1. Culture cells to the desired density (generally not to exceed 10^6 cells/mL for suspension cells).
2. Induce apoptosis or mitochondrial depolarization according to your specific protocol. Include an uninduced control sample.
3. For adherent cells, remove cells from the substrate to generate a single-cell suspension before staining. Transfer 0.5 mL of the cell suspension to a centrifuge tube.
4. Pellet cells by centrifugation for 5 minutes at room temperature at 400 x g.
5. Remove the supernatant.
6. Resuspend cells in 0.5 mL of Aquaphile™ JC-1 staining solution.
7. Incubate the cells in a 37°C cell culture incubator for 15 minutes.
8. Centrifuge cells for 5 minutes at 400 x g and remove the supernatant.
9. Optional: Wash the cells by resuspending the cell pellet in 2 mL pre-warmed PBS or cell culture medium followed by centrifugation. Remove the supernatant.
10. Resuspend the cell pellet in 0.5 mL PBS or cell culture medium.
11. Analyze cells by flow cytometry immediately following staining. Red JC-1 aggregates are detectable in the PE or PI channel (FL2), and green JC-1 monomers are detectable in the FITC channel (FL1). See instrument setup guidelines below.

Instrument Setup for Two Parameter Analysis by Flow Cytometry

Note: See Considerations for Staining and Reference 1 for notes on optimal excitation wavelength.

1. Run the uninduced control sample first. Generate a log FL1 (x-axis) versus log FL2 (y-axis) dot plot. Add quadrants to the dot plot.
2. Adjust FL1 and FL2 PMT voltages to register a dual-positive population in the upper-right quadrant with the dual-positive population falling within the second and third log decade scale of FL1 and FL2.
3. The upper-right quadrant borders should be adjusted so that the statistics read >95% on gated events.

   Note: 95% population gating assumes that the cells are healthy with high mitochondrial membrane potential. The actual number may vary depending on the state of the cells.
4. Run the induced sample using the PMT settings established above for the uninduced control sample. One should see a population of cells that appears in the lower-right quadrant of the graph. This reflects a loss of red emission on the FL2 axis.
5. If the induced sample exhibits only a minimal decrease in red emission, increase the FL2 - %FL1 compensation, and then repeat steps 3 and 4. Repeat compensation adjustment as needed to optimize discrimination between the uninduced and induced cell populations in FL2.

Staining Protocol for Fluorescence Ratio Detection by Microplate Reader

Note: JC-1 fluorescence is localized intracellularly, not distributed homogeneously throughout the medium. Different microplate readers may have higher or lower sensitivity for cellular fluorescence, particularly in adherent cells. If options for bottom read vs. top read, focal height adjustment, and/or well scanning are available on your reader, you may need to use these to optimize the detection settings for cells. Note that clear-bottom plates are required for bottom read on a fluorescence microplate reader.

1. Plate cells in a 96-well plate and grow to the desired density.
2. Induce apoptosis or depolarization according to your specific protocol. We recommend that you include an uninduced control sample.
3. Stain cells according to the staining protocol for fluorescence microscopy.
4. Measure red fluorescence (Ex/Em 550/600 nm) and green fluorescence (Ex/Em 485/535 nm) using a fluorescence microplate reader.

   Note: Use the closest settings available on your instrument. Note that these recommended excitation wavelengths are lower than the Ex maxima for the dye to reduce background from excitation light.
5. Determine the ratio of red fluorescence divided by green fluorescence.

   Note: The ratio of red to green fluorescence is decreased in cells with depolarized mitochondria (dead cells and cells undergoing apoptosis) compared to healthy cells. See References 2-3.

Related Products

Cat. No.	Product
10402-10403, 10405-10408	NucView® Caspase-3 Substrates (1 mM in PBS or DMSO)
70054... 70075	MitoView® Dyes
70082	MitoView® Fix 640
80011	Calcein AM
29003... 29085	Annexin V CF® Dye Conjugates
30063	CF®488A TUNEL Assay Apoptosis Detection Kit
30064	CF®594 TUNEL Assay Apoptosis Detection Kit
30074	CF®640R TUNEL Assay Apoptosis Detection Kit
30066	Apoptotic, Necrotic, Healthy Cells Kit Plus
30025	Resazurin Cell Viability Assay Kit (alamarBlue®)
70016	TMRE
70005	TMRE, 2 mM in DMSO
70017	TMRM

Please visit our website at www.biotium.com for information on our life science research products, including environmentally friendly EvaGreen® qPCR master mixes, fluorescent CF® Dye antibody conjugates and reactive dyes, apoptosis reagents, fluorescent probes, and kits for cell biology research.