

# Product Information

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## Mitochondria Staining Kit For mitochondrial potential changes detection

Catalog Number **CS0390**

Storage Temperature 2–8 °C

## TECHNICAL BULLETIN

### Product Description

The dissipation of the mitochondrial electrochemical potential gradient ( $\Delta\psi$ ) is known as an early event in apoptosis. This kit offers a fast and convenient method for the detection of changes in mitochondrial inner-membrane electrochemical potential in living cells using the cationic, lipophilic dye, JC-1 (5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolcarbocyanine iodide), and therefore, is suitable for apoptotic cell detection. Uptake by the mitochondria of JC-1 may be utilized as an effective distinction between apoptotic and healthy cells.

In normal cells, due to the electrochemical potential gradient, the dye concentrates in the mitochondrial matrix, where it forms red fluorescent aggregates (J-aggregates). Any event that dissipates the mitochondrial membrane potential prevents the accumulation of the JC-1 dye in the mitochondria and thus, the dye is dispersed throughout the entire cell leading to a shift from red (J-aggregates) to green fluorescence (JC-1 monomers).

This kit contains valinomycin that permeabilizes the mitochondrial membrane for  $K^+$  ions, and thus, dissipates the mitochondrial electrochemical potential and may be used as a control that prevents JC-1 aggregation.

The fluorescence of the cells stained with this kit may be observed by fluorescence microscopy or measured by fluorimetric and flow cytometry assays.

### Components

The kit is sufficient for 40 tests of 5 ml cell suspension or 200 tests of 1 ml cell suspension

5,5',6,6'-Tetrachloro-1,1',3,3'-Tetraethylbenzimidazolcarbocyanine Iodide (JC-1) Catalog Number T4069	1 mg
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DMSO Catalog Number D8418	1 ml
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JC-1 Staining Buffer 5× Catalog Number J3645	120 ml
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Valinomycin Ready Made Solution (1 mg/ml) Catalog Number V3639	0.1 ml
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### Reagents and Equipment Required but Not Provided

- Ultrapure water
- Centrifuge
- Fluorescence microscope
- Flow cytometer
- Fluorimeter

### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices. Valinomycin is highly toxic. Avoid contact of valinomycin with the skin and eyes.

### Storage

The kit should be stored at 2–8 °C. Upon reconstitution of the JC-1 dye, the 200× JC-1 Stock Solution should be stored at –20 °C.

### Preparation Instructions

Read the entire Procedure Section before preparing and storing the stock solutions. Different volumes of the prepared reagents are required for staining cells in suspension compared to adherent cells. The nature and amount of cells to be stained determine the volume of reagents required.

**200× JC-1 Stock Solution (1 mg/ml)** - The JC-1 dye is supplied as a lyophilized powder. Add 200 µl of DMSO (Catalog Number D8418) from the bottle supplied with the kit to the vial containing the JC-1 (Catalog Number T4069). Close the vial firmly and vortex it. Leave the vial at room temperature for ~15 minutes to ensure the JC-1 is completely dissolved. Transfer the JC-1 solution into the original DMSO bottle (Catalog Number D8418) containing the remaining solvent. Mix the solution and store at -20 °C in working aliquots. For a given procedure, it is important that all the samples (including the control) are stained with the same aliquot of the 200× JC-1 Stock Solution.

**1× JC-1 Staining Buffer** - The staining procedures require the use of both the JC-1 Staining Buffer 5× (Catalog Number J3645) and a prepared 1× JC-1 Staining Buffer. Therefore, **do not** dilute the entire volume of the JC-1 Staining Buffer 5× at once. Read the entire Procedure Section to determine the volume of 1× JC-1 Staining Buffer required. The 1× JC-1 Staining Buffer is prepared by a 5-fold dilution of the JC-1 Staining Buffer 5× (Catalog Number J3645) with ultrapure water.

### Procedure

#### A. Staining of Cells in Suspension

The recommended density of cells in suspension, for most cell lines, is  $0.2\text{--}1.2 \times 10^6$  cells/ml. The optimal cell density for each individual cell line needs to be determined. The cell density parameter is not relevant for adherent cells.

The following procedure is for 5 ml of a cell suspension. The procedure can be scaled up or down.

#### 1. Prepare Staining Solution.

Mix 25 µl of the 200× JC-1 Stock Solution in 4 ml of ultrapure water in an appropriate tube. Close the tube and mix the solution by inversion or vortex the tube briefly. Incubate the tube for 1–2 minutes at room temperature to ensure the JC-1 is completely dissolved. Open the tube and add 1 ml of the JC-1 Staining Buffer 5×. Mix by inversion.

**Note:** For a valinomycin control (mitochondrial gradient dissipation), add 1 µl of the Valinomycin Ready Made Solution (Catalog Number V3639) to the staining solution. Mix well.

2. Mix 5 ml of the cell suspension ( $0.2\text{--}1.2 \times 10^6$  cells per ml) in the complete medium (the medium used for cell growth containing the required supplements) with 5 ml of the Staining Solution prepared in step 1.
3. Incubate for 20 minutes at 37 °C in a humidified atmosphere containing 5% CO<sub>2</sub>.
4. While incubating, prepare 10 ml of the 1× JC-1 Staining Buffer by diluting 2 ml of the JC-1 Staining Buffer 5× with 8 ml of water. Place the tube on ice.
5. Centrifuge the cell suspension at 600 x g for 3–4 minutes at 2–8 °C. Aspirate the supernatant and place the tube with the cell pellet on ice.
6. Wash the cells with 5 ml of the ice-cold 1× JC-1 Staining Buffer and then resuspend the cells in 5 ml of the ice-cold 1× JC-1 Staining Buffer.

**Note:** At this step the cells can be concentrated or further diluted as required for a downstream application.

The stained cells may be observed by fluorescence microscopy or measured by fluorimetric and flow cytometry assays. **The sample should be kept on ice and analyzed as soon as possible, definitely no longer than 30 minutes after staining.**

#### B. Staining of Adherent cells

1. Prepare the Staining Solution as described in step A1.
2. Prepare a Staining Mixture by mixing the Staining Solution (step B1) with an equal volume of the complete medium for cell growth.
3. Aspirate the growth medium from the flask and overlay the cells with the Staining Mixture (step B2). Add 0.2–0.4 ml of the Staining Mixture per 1 cm<sup>2</sup> of growth surface.
4. Incubate the cells for 20 minutes at 37 °C in a humidified atmosphere containing 5% CO<sub>2</sub>.
5. Aspirate the Staining Mixture. Wash the cells twice with growth medium. Overlay the cells with the growth medium and observe the cells under a fluorescence microscope.

**Note:** Phenol red does not interfere with JC-1 staining.

### C. Fluorescence Determination

The principle of all the following detection methods is a comparison between the red fluorescence of the aggregated JC-1, representing intact mitochondria, to the green fluorescence of the monomeric JC-1, which represents disrupted mitochondria.

#### 1. Fluorimetry

For JC-1 monomers, set the fluorimeter at 490 nm excitation wavelength and 530 nm emission wavelength and measure the fluorescence. For JC-1 aggregates, set the fluorimeter at 525 nm excitation wavelength and 590 nm emission wavelength and measure the fluorescence.  
Note: The optimal excitation wavelength for the JC-1 aggregates is 525 nm, but excitation at 490 nm may be used.

#### 2. Fluorescence microscopy

JC-1 aggregates can be visualized in the mitochondria (bright red) using standard broad-pass filters that are used routinely for propidium iodide or Cy<sup>TM</sup>3 visualization. JC-1 monomers are visualized with broad-pass filters that are routinely used for the green fluorescent compounds like FITC. Dual band-pass filters designed to detect two fluorescent probes simultaneously (for example FITC/Cy3) can also be used.  
Note: Red JC-1 fluorescence fades faster than the green. Take an image of the stained cells in the red channel first.

#### 3. Flow cytometry

JC-1 monomers should be detected in the FL1 channel. JC-1 aggregates should be detected in FL2 channel.

### **References**

1. Reers, M., et al., J-aggregate formation of a carbocyanine as a quantitative fluorescent indicator of membrane potential. *Biochemistry*, **30**, 4480-4486 (1991).
2. Cossarizza, A., et al., New method for the cytofluorimetric analysis of mitochondrial membrane potential using the J-aggregate forming lipophilic cation 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolcarbocyanine iodide (JC-1). *Biochem. Biophys. Res. Commun.*, **197**, 40-45 (1993).
3. Smiley, S.T., et al., Intracellular heterogeneity in mitochondrial membrane potentials revealed by a J-aggregate-forming lipophilic cation JC-1. *Proc. Natl. Acad. Sci. USA*, **88**, 3671-3675 (1991).
4. Inai, Y., et al., Valinomycin induces apoptosis of ascites hepatoma cells (AH-130) in relation to mitochondrial membrane potential. *Cell Struct. Funct.*, **22**, 555-563 (1997).

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EB/MAM 02/08-1

## Troubleshooting guide

Problem	Cause	Solution
<b>In the Red Channel</b>		
No shift (or less than expected) in the red fluorescence upon valinomycin treatment or apoptosis induction of cells	JC-1 was not completely dissolved. 200× JC-1 Stock Solution was diluted directly in the 1× JC-1 Staining Buffer or added directly to cells.	Dilute the 200× JC-1 Stock Solution with ultrapure water only (see step A1).
	Cells under starvation conditions	Use viable, well dividing cells in the mid-log growth phase.
	Cell wash was not sufficient.	Use <b>ice-cold</b> 1× JC-1 Staining Buffer for cell wash. Washing with a warm Staining Buffer is much less effective.
Red debris observed under the microscope.	JC-1 was not completely dissolved.	Dilute the 200× JC-1 Stock Solution with ultrapure water only (see step A1).
	Cell wash was not sufficient.	Use <b>ice-cold</b> 1× JC-1 Staining Buffer for cell wash. Washing with a warm Staining Buffer is much less effective.
The cell plasma membrane is stained with red patches.	Overgrown cells or cells under starvation conditions	Use viable, well dividing cells in the mid-log growth phase.
<b>In the Green Channel</b>		
No shift (or less than expected) in the green fluorescence upon valinomycin treatment or apoptosis induction of cells	The time interval between the staining and the analysis was too long.	Analyze the cells immediately after staining.
	Cells were kept warm before analysis.	Keep the stained/washed cells on ice before the analysis.
<b>General</b>		
Overall fluorescence is weak.	JC-1 was not completely dissolved.	Dilute the 200× JC-1 Stock Solution with ultrapure water only (see step A1).
	Photobleaching of JC-1	JC-1 in aqueous solution is light sensitive. Do not expose the JC-1 Staining Solution and stained cells to a bright light.
Inconsistent results	Different cell growth/treatment conditions between the tested and the control samples.	Use cells in the same growth phase (from the same flask if possible) and an equal number of cells for a particular experiment and its controls. Apoptosis inducing agents may impede cell growth. Normalize cell numbers if needed.
	Different aliquots of 200× JC-1 Stock Solution were used in the same experiment.	Use a single aliquot of the 200× JC-1 Stock Solution for the entire experiment. If more than one aliquot is required for a single experiment, pool aliquots of 200× JC-1 Stock Solution.

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