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Product Information

JC-1 Mitochondrial Membrane Potential Detection Kit

Kit Contents

Component	30001-T 25 assays	30001 100 assays
JC-1, 100X in DMSO	30001-TA 125 uL	30001A 500 uL
10X Assay Buffer	30001-TB 2.5 mL	30001B 10 mL

Assay number based on flow cytometry protocol. Actual number of assays may vary by application.

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

Product Description

The loss of mitochondrial membrane potential ($\Delta\Psi$) is a hallmark of apoptosis. It is an early event preceding phosphatidylserine externalization and coinciding with caspase activation (1,2). During mitochondrial permeability transition, the electrochemical gradient across the mitochondrial membrane collapses. The collapse is thought to occur through the formation of pores in the mitochondria by dimerized Bax or activated Bid, Bak, or Bad proteins. Activation of these pro-apoptotic proteins is accompanied by the release of cytochrome c into the cytoplasm (3-6).

The JC-1 assay uses a unique cationic dye (5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolylcarbocyanine iodide) to detect loss of mitochondrial membrane potential (7). The negative charge within mitochondria created by the mitochondrial membrane potential allows the lipophilic dye, bearing a delocalized positive charge, to enter the mitochondrial matrix where it accumulates. When the critical concentration is exceeded, J-aggregates form, which fluoresce red (8). JC-1 also localizes in the cytosol in its green fluorescent monomeric form, thereby staining healthy cells with both red and green fluorescence. In apoptotic cells, the mitochondrial membrane potential collapses, and JC-1 cannot accumulate in mitochondria. In these cells, JC-1 remains in the cytoplasm in a green fluorescent monomeric form. Apoptotic cells, showing primarily green fluorescence, are easily differentiated from healthy cells, which show red and green fluorescence. The aggregate red form has absorption/emission maxima of 585/590 nm (7). The green monomeric form has absorption/ emission maxima of 510/527 nm.

The JC-1 assay is fast and simple, and can be analyzed by flow cytometry, fluorescence microscopy, or fluorescence microplate reader (9,10).

References

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8. Cossarizza, A., et al. Biochem. Biophys. Res. Commun. 197 (1): 40-45 (1993).
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Assay Protocols

Reagent preparation

Preparation of 1X Assay Buffer

Note: use of Assay Buffer is optional, you can also perform JC-1 staining in the buffer or cell culture medium of your choice.

1. If necessary, warm the 10X Assay Buffer until any salt crystals are completely dissolved.
2. Dilute the Assay Buffer 1:10 with dH₂O (e.g. 1 mL 10X assay buffer + 9 mL dH₂O).

Preparation of JC-1 Reagent working solution

Immediately before use, dilute the 100X JC-1 reagent to 1X by adding 10 uL of 100X JC-1 per mL of 1X Assay Buffer or pre-warmed media of your choice to generate JC-1 Reagent working solution.

Staining protocol for flow cytometry

Cell staining

1. Culture cells to the desired density (generally not to exceed 10⁶ cells/mL for suspension cells).
Note: each cell line should be evaluated on an individual basis to determine optimal cell density for apoptosis induction.
2. Induce apoptosis 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 cell suspension to a centrifuge tube.
4. Pellet cells by centrifugation for 5 minutes at room temperature at 400 xg.
5. Remove the supernatant.
6. Resuspend cells in 0.5 mL 1X JC-1 Reagent working solution.
7. Incubate the cells in a 37°C cell culture incubator for 15 minutes.
8. Centrifuge for 5 minutes at 400 xg and remove supernatant.
9. Wash the cells by resuspending the cell pellet in 2 mL PBS or cell culture medium followed by centrifugation. Remove the supernatant. Repeat once.
10. Resuspend the cell pellet in 0.5 mL PBS or cell culture medium. Cells are now ready for flow cytometry analysis.

Quantification by flow cytometry

Analyze cells immediately following step 10 by flow cytometry. Mitochondria containing red JC-1 aggregates in healthy cells are detectable in the PE or PI channel (FL2), and green JC-1 monomers in apoptotic cells are detectable in FITC channel (FL1).

Instrument set-up for two parameter analysis

1. Run the uninduced control sample first. Generate a log FL1 (X-axis) versus log FL2 (Y-axis) dot plot. Add regions R2 and R3 to the dot plot.
2. Adjust FL1 and FL2 PMT voltages to register a dual positive population in region 2 (R2). The peak of the dual positive population should fall within the second and third log decade scale of FL1 and FL2.
3. The region 2 (R2) gate should be adjusted to include >95% of events. This number will vary depending on the condition 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 region 3 (R3). This reflects a loss of red emission on the FL2 axis, which corresponds to the loss of mitochondrial membrane potential in induced cells.
5. If the induced sample exhibits only a minimal decrease in red emission, increase the FL2 - %FL1 compensation.
6. Repeat steps 3 and 4.
See Tech Note 1: Quadrants

Continued next page.

Staining protocol for fluorescence microscopy

Staining of cells in suspension

1. Stain cells according to the protocol for flow cytometry.
2. Resuspend the cell pellet in 0.3 mL PBS or cell culture medium.

Staining of adherent cells

1. Grow cells to the desired density on a glass cover slip in a petri dish or in a chamber slide.
2. Induce cells according to your specific protocol. Include an uninduced control sample.
3. Remove the cell culture media and replace with JC-1 Reagent working solution sufficient to cover the cells.
4. Incubate the cells in a 37°C cell culture incubator for 15 min.
5. Remove media and wash once with PBS or cell culture medium.
6. Mount coverslips on a slide with a drop of PBS or cell culture medium. For chamber slides, add PBS or cell culture medium sufficient to cover the cells.

Imaging by fluorescence microscopy

Observe cells immediately after staining with a fluorescence microscope using a dual band-pass filter designed to simultaneously detect fluorescein and rhodamine or fluorescein and Texas Red®. In cells with intact mitochondrial membrane potential, the mitochondria will fluoresce red with emission at 590 nm, and the cytoplasm will fluoresce green. In apoptotic and dead cells, the dye will remain in its monomeric form and will appear green with an emission at 530 nm. (See Tech Note 2: Filters for fluorescence microscopy).

Staining protocol for fluorescence ratio detection (see refs. 7-10)

1. Plate cells in a 96-well plate and grow to the desired density.
2. Induce cells according to your specific protocol. Include an uninduced control sample.
3. Stain cells according to the staining protocol for fluorescence microscopy
4. Measure red fluorescence (excitation 550 nm, emission 600 nm) and green fluorescence (excitation 485 nm, emission 535 nm) using a fluorescence microplate reader.
5. Determine the ratio of red fluorescence divided by green fluorescence.
6. The ratio of red to green fluorescence is decreased in dead cells and in cells undergoing apoptosis compared to healthy cells.

Tech Notes

Tech Note 1: Quadrants

On instruments where it is not possible to add regions to the dot plot, quadrants can be added instead using the following protocol:

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 quadrant 2 with the dual positive population falling within the second and third log decade scale of FL1 and FL2.
3. The quadrant 2 markers should be adjusted so that the statistics read >95% on gated events.
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 4th quadrant. 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.
6. Repeat steps 3 and 4.

Tech Note 2: Filters for fluorescence microscopy

Red JC-1 aggregates and green monomers can be viewed simultaneously with a dual band-pass filter for fluorescein/rhodamine or fluorescein/Texas Red®. JC-1 aggregates can be imaged with a band-pass filter designed to detect rhodamine (Ex/Em: 540 /570 nm) or Texas Red® (Ex/Em: 590/610 nm). JC-1 monomers can be imaged with a band-pass filter used for the detection of fluorescein (Ex/Em: 490/520 nm).

Related Products

Catalog number	Product
70055	MitoView™ 633
70052	MitoView™ Blue
30029	NucView™ 488 Caspase-3 Assay Kit for live cells
30067	Dual Apoptosis Assay with NucView™ 488 Caspase-3 Substrate and CF™594 Annexin V
30062	NucView™ 488 and MitoView™ 633 Apoptosis Kit
10405	NucView™ 405 Caspase-3 Substrate
30019	MCB Glutathione Detection Kit
32002-32009	Live-or-Dye™ Fixable Viability Staining Kits (choose from 8 fluorescent dye colors)
30002	Viability/Cytotoxicity Assay Kit for Animal Live & Dead Cells
30026	Calcein AM Cell Viability Assay Kit
30025	Resazurin Cell Viability Assay Kit
30006	MTT Cell Viability Assay Kit
30007	XTT Cell Viability Assay Kit
30020	ATP-Glo™ Bioluminometric Cell Viability Assay Kit
30068	ViaFluor™ 405-SE Cell Proliferation Kit
30050	CFDA SE Cell Proliferation Assay Kit

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