ab113850 JC-1 – Mitochondrial Membrane Potential Assay Kit

For the measurement of mitochondrial membrane potential by fluorescence plate reader. This product is for research use only and is not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab113850
(use abcam.cn/ab113850 for China, or abcam.co.jp/ab113850 for Japan)

Materials Supplied and Storage

Store kit as described below immediately upon receipt.
Lyophilized JC-1 is stable for 12 - 18 months if stored in the dark at -20°C. Once JC-1 is reconstituted in DMSO, aliquot and store at -20°C.
Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 4.

Item	Amount	Storage Condition (Before Preparation)
JC-1 (Lyophilized)	500 µg	-20°C
10X Dilution Buffer (sterile)	10 mL	+4°C
DMSO (cell culture tested)	1 mL	-20°C
50 mM FCCP (Carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone)	10 µL	-20°C

Materials Required, Not Supplied:

- Fluorescence plate reader. JC-1 may also be detected with similar settings to those used to detect rhodamine (excitation/emission wavelengths: 540/570 nm) or texas red (excitation/emission wavelengths: 590/610 nm)
- General tissue culture supplies
- PBS (1.4 mM KH2PO4, 8 mM Na2HPO4, 140 mM NaCl, 2.7 mM KCl, pH 7.4)
- Fetal Bovine Serum (FBS)
- Sterile, tissue culture treated, clear bottom, dark sided 96-well microplates
- Multichannel pipette (50 – 300 µL)
- Optional:
  - Test compounds/diluents of interest
  - Uncouplers include CCCP (carbonyl cyanide 3-chlorophenylhydrazone), 2', 4' Dinitrophenol
  - 96-Well deep sided, clear bottom, dark sided microplates with lids

1. Reagent Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. The sample volumes below are sufficient for 96 100 µL tests; adjust volumes as needed for the number of wells used in your experiment.

1.1 1X Dilution Buffer: Prepare 1X Dilution Buffer by adding 10 mL 10X Dilution Buffer to 90 mL deionized water. Mix gently and thoroughly.

1.2 1X Supplemented Dilution Buffer: Prepare 1X Supplemented Dilution Buffer by adding 2 mL FBS to 18 mL 1X Dilution Buffer.

1.3 1 mM JC-1 Stock Solution: Allow the lyophilized vial JC-1 and the DMSO to warm to room temperature. Reconstitute the JC-1 by adding 766.8 µL DMSO to the 500 µg vial of JC-1. *Any unused 1 mM JC-1 solution should be aliquoted and stored at -20 °C.*

1.4 Working JC-1 Solution: To prepare the Working JC-1 Solution, add the appropriate volume of 1 mM JC-1 Solution to previously warmed 1X Dilution Buffer. As an example, to generate a 1 µM JC-1 Solution, mix 10 µL 1 mM JC-1 with 10 mL of 1X Dilution Buffer. Mix thoroughly and gently. If necessary, centrifuge the 1 µM solution at 13,000 x g for 3 minutes to sediment non-soluble particles.
The exact concentration of JC-1 required will depend on the cell line being used and must be determined on an individual basis by the end user. Typical working concentrations, along with recommended seeding densities (steps 4.1.6 & 4.2.2) for certain cell lines are shown in the table below:

Typical working concentrations		
Sample Type	**Seeding Density per Well**	**JC-1 Concentration (µM)**
HepG2	$1.5x10^4$	20
HL60	200,000	10
HeLa	6,000	1
HDFN	6,000	1

4. Assay Procedure

Equilibrate all materials and prepared reagents to 37°C prior to use. Assay all standards, controls and samples in duplicate.

4.1 Fluorescent Microplate Measurement (Suspension Cells, e.g. HL60 cells)

4.1.1 Grow HL60 cells in glucose based media so that approximately $2.5x10^7$ cells are available on the day of the experiment per plate

4.1.2 If performing toxicity assays, dilute compounds of interest in 1X supplemented dilution buffer to 2X of final desired concentration for the experiment. A 96-well deep well microplate may be use in this step. Compounds may also be diluted in complete media with 10% FBS without phenol red. Include a depolarization control (100 µM FCCP) and a normal control (vehicle or diluent of choice).

4.1.3 Collect cells and wash by centrifugation once in 1X Dilution Buffer or 1X PBS.

4.1.4 Resuspend cells in 10 mL of the Working JC-1 Solution and incubate at 37°C for 30 minutes in the dark.

4.1.5 Wash cells by centrifugation with 10 mL of 1X Dilution Buffer.

4.1.6 Resuspend $2x10^7$ cells in 5 mL of 1X Supplemented Dilution Buffer.

4.1.7 Seed a 96-well dark plate as follows: 200,000 stained cells/50 µL/well. Include blank wells (with non-stained cells).

4.1.8 If performing toxicity assays, add to each well 50 µL of previously diluted 2X compounds and treat for desired period of time

4.1.9 Read plate end point in the presence of compounds, media or buffer on a fluorescent plate reader. Set excitation wavelength at 535 ± 17.5 (aggregate excitation only) or 475 ± 20 nm (for simultaneous aggregate and monomer excitation). Set emission wavelength at 590 ± 17.5 nm (aggregate emission only). If reading of the monomer species is also desired, set a second emission reading at 530 ± 15 nm.
FCCP 100 µM treatment for 4 hours should decrease the JC-1 aggregate signal to at least 25-30% from control levels.

4.2 Fluorescent Microplate Measurement (Adherent Cells, e.g. HepG2 cells)

4.2.1 Grow HepG2 cells in standard media so that $3x\ 10^6$ to $4x\ 10^6$ cells are obtained the day before the experiment per plate.

4.2.2 Harvest cells the day before the experiment and seed a dark 96-well microplate with $1.5x10^4$ cells per well in standard growing culture media. Allowed to attach overnight.

4.2.3 If performing toxicity assays, dilute compounds of interest to the final desired concentration in 1X Supplemented Dilution Buffer solution. A 96-well deep well microplate may be use in this step. Compounds may also be diluted in complete media with 10% FBS without phenol red. Include positive (100 µM FCCP) and negative controls (vehicle of choice)

4.2.4 Wash the HepG2 cells seeded on the 96-well plate with 100 µL/well of 1X Dilution Buffer or 1X PBS once.

4.2.5 Add 100 µL/well of the Working JC-1 Solution and incubate for 10 minutes at 37°C in the dark. Include blank wells (with non-stained cells).

4.2.6 Wash the plate twice with 1X Dilution Buffer solution.

4.2.7 If performing cytotoxicity assays, add compounds of interest and treat for desired period of time.

4.2.8 Read plate end point in the presence of compounds, media or buffer on a fluorescent plate reader. Set excitation wavelength at 535 ± 17.5 nm (aggregate excitation only) or 475 ± 20 nm (for simultaneous aggregate and monomer excitation). Set emission wavelength at 590 ± 17.5 nm (aggregate emission only). If reading of the monomer species is also desired, set a second emission reading at 530 ± 15 nm.

4.2.9 FCCP 100 µM treatment for 4 hours should decrease the JC-1 aggregate signal to at least 25-30% from control levels.

4.2.10 Note: Buffer or compound must be present in the wells during the reading of the signal. Do not allow wells to dry out

Calculations

Subtract background (590nm emission of non-stained cells for aggregated JC-1 and 535nm emission of non-stained cells for monomeric JC-1) from test signals. Express signal as percentage of control (untreated healthy cells). If both monomer and aggregate forms are measured, a ratio between the two measurements may be obtained and plotted. Data obtained with the JC-1 assay gives a relative measure of mitochondrial membrane potential as a percentage of control and cannot be used for absolute measurements of membrane potential in millivolts. Decrease in JC-1 signal may indicate either mitochondrial depolarization or cell death and must be interpreted in parallel with a cytotoxicity assay (such as the ATP detection kit ab113849). The data in Figure 1 below shows the uncoupling effect of FCCP acute treatment on HL60 cells as measured with the JC-1 stain and read on a fluorescent plate reader.

FAQs

Q. Is this kit designed to do a cell treatment before or after JC-1 incubation?

A. It is essential to read the JC-1 signal in the presence of compound (or treatment) because changes in membrane potential can be short lived and reversible as soon as the treatment is removed from the cells

Q. I am already growing cells on coverslips, will I be able to use these cells with the assay?

A. Any mammalian live cell may be stained with JC-1 provided that the staining concentration has been optimized. When cells are seeded on coverslips, two major changes must be done to the protocol in order to obtain the correct results:

- Volumes will have to be adjusted from 100µL per well to 500 - 100 µL per well depending on whether the cells are seeded on a 6-well plate or on a 24-well plate. Due to the change in volume, only 10 to 20 tests can be run using this kit under these conditions.
- Coverslips must be ideally imaged on an inverted fluorescence microscope or a microscope with a lens that can be immersed in water, using a Texas Red filter. In the absence of these instruments, the coverslip could be mounted with the buffer provided on top of an imaging chamber gasket. In this later case, imaging must be prompt to prevent bleaching of the dye.

Q. I am using primary cells and I'm afraid that 10 minutes without media is too much. Can I mix the JC-1 with the growing meda rather than the supplied buffer?

A. Yes, the JC-1 may be mixed with the growing media. However, caution must be taken as the phenol red present in most media formulations may cause increased background. Furthermore, we have also observed higher background on RPMI media in comparison to DMEM media.

Q. Is fixation of the cells with paraformaldehyde in the wells possible, after live imaging, for storage purposes?

A. Fixation of the cells will interfere with the dye signal. Once the signal has been obtained by live imaging or fluorescent read out, cells cannot be stored for future use.

Q. I am planning a drug treatment for 18 hours; can I run FCCP in parallel for 18 hours? Will the signal dissipate after 4 hours?

A. We do not recommend treating cells with FCCP for more than 4 hours as this will generate excessive toxicity and results will represent depolarization but rather general cell toxicity. Furthermore, incubations for longer than 4 hours after JC-1 staining may lead to dissipation of the signal. In this case, we suggest reversing the protocol and treat first prior to staining. The following protocol should be followed in this scenario:

- Dilute compounds of interest in complete media without phenol red. Make four times the volume required.
- Treat suspension or adherent cells for the desired period. If treating cells for microplate measurements, treat with 100 µL per well.
- Include blank wells with no cells but with compound at the same concentration used for treatment.
- Include at least 2 depolarized control wells, to be reserved for FCCP treatment, containing cells but none of the test compounds.
- 4 hours prior to completion of treatment, dilute FCCP to 10X of final concentration (1 mM) and spiked 10X FCCP into the reserved depolarized control wells by adding 11 µL per well.
- 1 hour prior to completion of the treatment, dilute JC-1 at 2X of the final concentration desired in the same media used for treatment (containing experimental compounds) and warm at 37°C.
- 10 – 30 minutes prior to completion of the treatment, overlay 2X JC-1 dilution on top of the treated cells. If treating cells for microplate measurements, overlay 100 µL of 2X JC-1 dilution per well.
- Incubate JC-1 and compounds for the desired period of time (10 – 30 minutes).
- Once incubation is completed, wash the wells twice with 100 µL per well of 1X Dilution buffer containing compounds. Leave last wash in the wells.
- Transfer the plate to the microplate reader and read according to the protocol.

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