# abcam

## Product datasheet

# JC-1 - Mitochondrial Membrane Potential Assay Kit ab113850

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#### Overview

**Product name** JC-1 - Mitochondrial Membrane Potential Assay Kit

**Detection method** Fluorescent

Sample type Adherent cells, Suspension cells

Assay time 1h 00m

Product overview JC1- Mitochondrial Membrane Potential Assay Kit ab113850 contains

tetraethylbenzimidazolylcarbocyanine iodide (JC-1), a cationic dye that accumulates in energized

mitochondria.

At low concentrations (due to low mitochondrial membrane potential), JC-1 is predominantly a monomer that yields green fluorescence with emission of 530±15 nm.

At high concentrations (due to high mitochondrial membrane potential), the dye aggregates yielding a red to orange colored emission (590±17.5 nm).

Therefore a decrease in the aggregate fluorescent count is indicative of depolarization whereas an increase is indicative of hyperpolarization.

The JC-1 staining protocol is very simple:

- wash cells in dilution buffer or PBS
- add JC solution
- incubate for 30 min at 37°C for suspension cells, or 10 min for adherent cells
- wash cells with dilution buffer
- treat cells as desired for experimental plan
- analyze on a fluorescent microplate reader

The aggregate dye can be excited at 535 nm, the monomer and aggregate together at 475 nm.

Review our <u>cell health assays guide</u> to learn more about our other <u>cell viability</u>, <u>cytotoxicity</u> and <u>cell proliferation</u> assay kits.

Review the **metabolism assay guide** to learn about assays for metabolites, metabolic enzymes, mitochondrial function, and oxidative stress, and also about how to assay metabolic function in live cells using your plate reader.

**Notes** 

#### **Properties**

#### Storage instructions

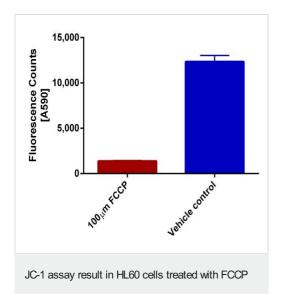
Store at -20°C. Please refer to protocols.

Components	100 tests
50mM FCCP (in DMSO)	1 x 10µl
Dilution Buffer (10X, sterile)	1 x 10ml
DMSO (cell culture tested)	1 x 1ml
JC-1 (lyophilized)	1 x 500µg

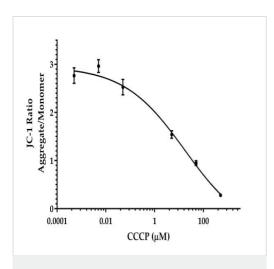
#### Relevance

Mitochondrial Membrane Potential is an important parameter of mitochondrial function used as an indicator of cell death. The collapse of the mitochondrial Membrane potential coincides with the opening of the mitochondrial permeability transition pores, leading to the release of cytochrome c into the cytosol, which in turn triggers other downstream events in the apoptotic cascade.

#### **Images**

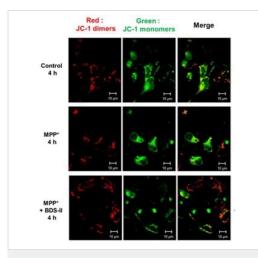


JC1 - Mitochondrial Membrane Potential Assay Kit (ab113850). HL60 cells were seeded and labeled according to section 11.1 of the protocol. Cells were then treated for 4 hours with 100  $\mu$ M FCCP or vehicle/diluent control (DMSO). Mean and standard deviation is plotted for 3 replicates from each condition.



JC-1 assay result in HepG2 cells treated with CCCP.

JC1 - Mitochondrial Membrane Potential Assay Kit (ab113850). HepG2 cells were seeded and labeled according to section 11.2 of the protocol. Cells were then treated for 4 hours with a titration series of CCCP (carbonyl cyanide 3-chlorophenylhydrazone) and both monomer and aggregate forms were read on a Perkin Elmer-Wallac 1420 Victor 2 Multilabel plate reader. Mean and standard deviation of aggregate/monomer ratios is plotted for 12 replicates for each concentration. IC50 of CCCP in HepG2 cells was calculated at 8.7  $\mu M$ .



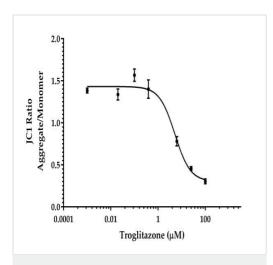
Using JC1 dye to examine mitochondrial membrane potential depolarization in SH-SY5Y cells

Image courtesy of Son MS et al. Sci Rep. 2017; 7: 2075. doi: 10.1038/s41598-017-02129-w. Reproduced under the Creative Commons License http://creativecommons.org/licenses/by/4.0/.

Son MS et al. (2017) used JC1 Mitochondrial Membrane Potential Assay Kit ab113850 to stain:

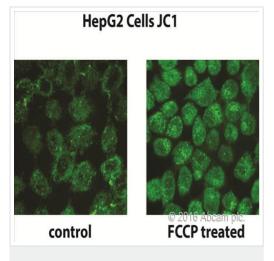
- untreated SH-SY5Y cells (control),
- SH-SY5Y cells treated with 1mM MPP+ (MPP+) and,
- SH-SY5Y cells pretreated with BDS-II followed by 1mM MPP<sup>+</sup> treatment (MPP<sup>+</sup> +BDSII).

Normal mitochondrial membrane potential is shown in red with JC-1 dimers and depolarized membrane potential is shown in green in JC-1 monomers.



JC-1 assay result in HL60 cells treated with Troglitazone

JC1 - Mitochondrial Membrane Potential Assay Kit (ab113850). HL60 cells were seeded and labeled according to section 11.1 of the protocol. Cells were then treated for 4 hours with a titration series of the thiazolidinedione Troglitazone and both monomer and aggregate forms were read on a Perkin Elmer-Wallac 1420 Victor 2 Multilabel plate reader. Mean and standard deviation of aggregate/monomer ratios is plotted for 3 replicates for each concentration. IC50 of Troglitazone in HL60 cells was calculated at  $1.2~\mu M$ .

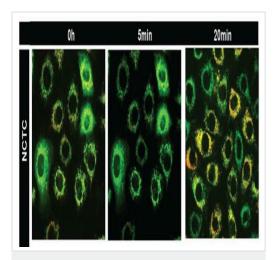


JC1 Mitochondrial Membrane Potential Assay Kit using green fluorescence imaging

This image is courtesy of an Abreview submitted by Dimitra Kalamida

The JC1- Mitochondrial membrane potential assay kit has been tested using HepG2 cells, control cells and FCCP-treated cells (100uM for 4h) have been used as a positive control. The company's instructions were followed for JC1 mitochondrial membrane potential assay. Imaging was performed on a customized Andor Revolution Spinning Disk Confocal System built around a stand (IX81 Olympus) with a 60x lens and a digital camera (Andor Ixon+885) (CIBIT Facility, MBG-DUTH). Image acquisition was performed in Andor IQ 2 software. Optical sections were recorded every 0.3 µm. All confocal microscopy images presented in this work are 2D maximum intensity projections of z-stack images (ImageJ 1.47v National Institute of Health,USA).

Personal feedback: A green laser with the appropriate emission filter (530nm) has been used to detect the monomer of the JC1 dye, following FCCP treatment the mitochondrial membrane potential of the cells was eliminated, as demonstrated by the increase of the monomer emission.



JC1 used to assay in vitro metabolic effect on

hepatocyte and hepatoma cells

Image courtesy of Koukourakis MI et al. Sci Rep. 2016; 6: 30986doi: 10.1038/srep30986. Reproduced under the Creative Commons License http://creativecommons.org/licenses/by/4.0/

Koukourakis MI et al. (2016) used the MMP assay kit to stain with JC-1 in NCTC hepatocytes exposed to amifostine (100  $\mu$ g/mI) over a time course of 20 minutes *in vitro*. Confocal microscopy used to assess mitochondrial membrane potential in the cells.

Serial images confirmed a rapid drop of both green and red fluorescence, one minute after exposure, an effect that was restored to normal at 20 minutes, after a small period of a rebound increase.

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