JC-1 MitoMP Detection Kit

Technical Manual

General Information

Mitochondria is one of the important organelles in discussing early cytotoxicity, oxidative stress and apoptosis in vivo[1]. Mitochondria synthesize ATP using oxygen to produce necessary energy for living cells. Lowering of mitochondrial activity and dysfunction are known to be closely related to cancer, aging, and neurodegenerative diseases such as Alzheimer's and Parkinson's diseases[2), 3)].

JC-1 is widely used for observing mitochondrial membrane potential, shows fluorescence characteristic change from green (530 nm) to red (590 nm) depending on the mitochondrial membrane potential.

A red/green fluorescence intensity ratio of JC-1 decreases in depolarized mitochondria due to disruption of red fluorescent J-aggregates.

Unlike commercially available JC-1, no precipitation of JC-1 in aqueous buffer occurs by using this kit. In addition, the Imaging Buffer including in this kit minimizes fluorescence background and maintains the cells being healthy during assays.

Kit Contents

JC-1 Dye	100 nmol x 1
Imaging Buffer (10x)	6 mL x 1

Storage Condition

Store at 0-5 °C.

Required Equipment and Materials

- Dimethyl sulfoxide (DMSO)
- Medium or HBSS
- Micropipettes
- Microtube

Preparation of Solutions

Preparation of 1-2 mmol/L of JC-1 DMSO stock solution

Add appropriate volume of JC-1 DMSO to a tube of JC-1 Dye according to the Table 1 below and dissolve the red-purple solid by pipetting. Store the JC-1 DMSO stock solution at -20 °C.

*Protect the JC-1 DMSO stock solution from light.

*JC-1 DMSO stock soltuion is stable at -20°C for up to a month.

Table 1 Preparation guide to prepare JC-1 DMSO stock solution

JC-1	DMSO
1 mmol/L	100 µL
2 mmol/L	50 µL

Preparation of 1-15 µmol/L JC-1 working solution

Take a necessary amount of the JC-1 DMSO stock solution with a micropipette and transfer it to a microtube.
Add appropriate amount of medium to the tube, then mix immediately by 10 times of pipetting.
(Example) To prepare 2 µmol/L JC-1 working solution, transfer 2 µL of JC-1 DMSO stock solution to a microtube.
Add 1 mL of medium to the tube, then mix immediately by 10 times of pipetting.

*To prepare a homogeneous working solution, please exactly follow the preparing procedure above.

*Equilibrate both DMSO stock solution and culture medium to room temperature before preparing a working solution.

*To get more fluorescence intensity, please consider the concentration of JC-1 working solution and Incubate time.

*Please use the JC-1 working solution within the day.

Preparation of Imaging Buffer solution

Dilute Imaging Buffer (10x) 10 times using double-deionized water.

*Please prepare appropiate amount of Imaging Buffer solution and use the solution within the day.

General protocol

JC-1 staining

1. Inoculate cells into a dish or a chamber slide and incubate the cells at 37 °C in a 5% CO_2 incubator.
2. Add an appropriate volume of JC-1 working solution to wells.
3. Incubate the cells at 37 °C in the 5% CO_2 incubator for 30-60 minutes.
4. Discard the supernatant and wash the cells with HBSS twice.
5. Add Imaging Buffer solution and observe the cells under a fluorescence microscope.

Fluorescence microscopic detection of mitochondrial membrane potential in HeLa cells treated with Carbonyl cyanide m-chlorophenyl hydrazone (CCCP)

1. HeLa cells(2.4×10^5 cells/mL, 200 µL) in MEM (10% fetal bovine serum, 1% penicillin-streptomycin) were seeded on a µ-slide 8 well plate (ibidi) and were cultured at 37 °C in a 5% CO_2 incubator overnight.
2. The medium was removed and CCCP in MEM (0 or 100 µmol/L, 200 µL) was added to each well, and then the cells were cultured at 37 °C for 90 minutes in the 5% CO_2 incubator.
3. After the supernatant (100 µL) was removed, JC-1 working solution (4 µmol/L, 100 µL) was added, then the cells were cultured at 37 °C for 30 minutes in the 5% CO_2 incubator.
4. The cells were washed with 200 µL of HBSS twice.
5. Imaging Buffer solution (200 µL) was added, and the cells were observed under a fluorescence microscope.

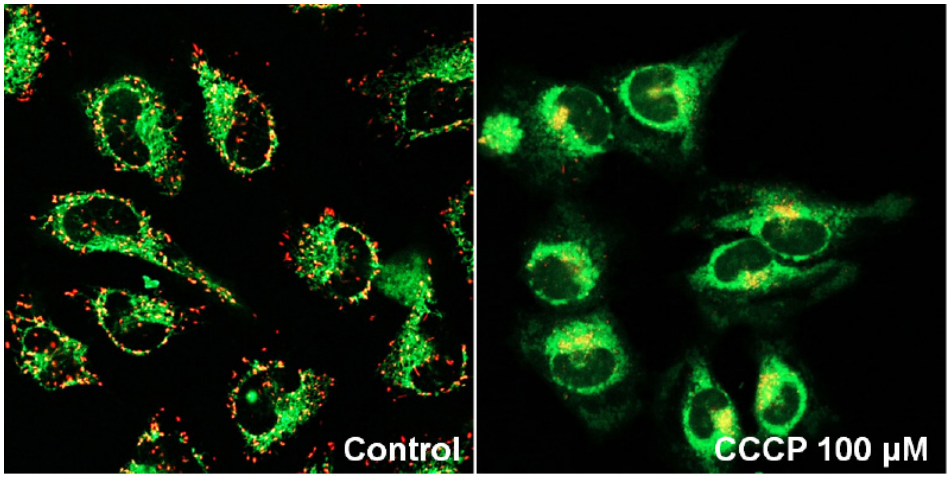


Figure 1 Fluorescence imaging of mitochondrial membrane potential in HeLa cells

<Imaging Filter>
Green : 488 nm (Ex), 500-550 nm (Em), Red : 561 nm (Ex), 560-610 nm (Em)

Fluorescence microscopic detection of mitochondrial membrane potential in HeLa cells treated with Carbonyl cyanide-p-trifluoromethoxyphenyl hydrazone (FCCP)

1. HeLa cells(2.4×10^5 cells/mL, 200 µL) in MEM (10% fetal bovine serum, 1% penicillin-streptomycin) were seeded on a µ-slide 8 well plate (ibidi) and were cultured at 37 °C in a 5% CO_2 incubator overnight.
2. The medium was removed and FCCP in MEM (0 or 100 µmol/L, 200 µL) was added to each well, and then the cells were cultured at 37 °C for 30 minutes in the 5% CO_2 incubator.
3. After the supernatant (100 µL) was removed, JC-1 working solution (4 µmol/L, 100 µL) was added, then the cells were cultured at 37 °C for 30 minutes in the 5% CO_2 incubator.
4. The cells were washed with 200 µl of HBSS twice.
5. Imaging Buffer solution (200 µL) was added, and the cells were observed under a fluorescence microscope.

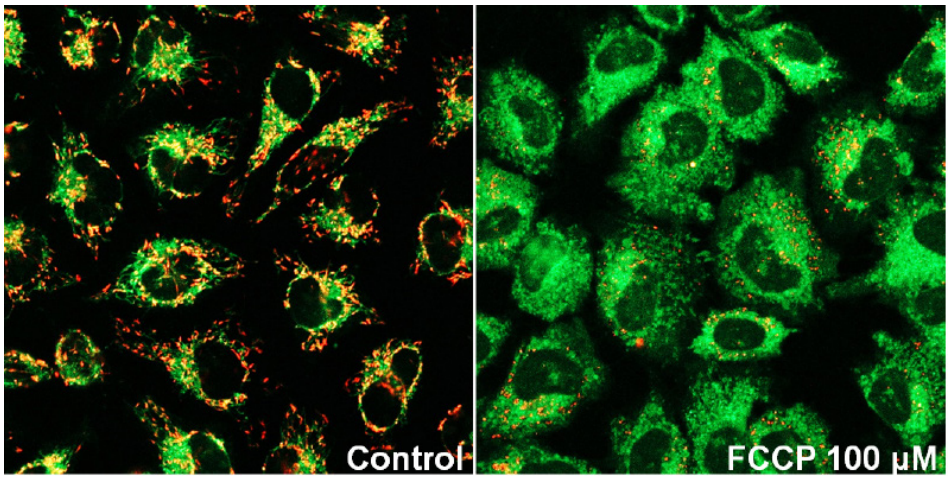


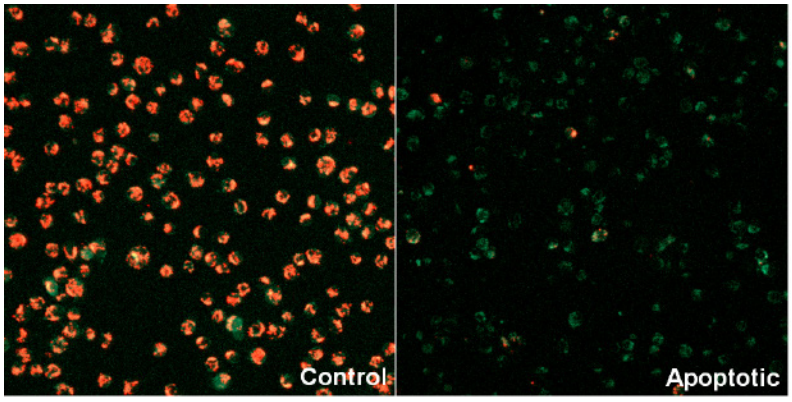
Figure 2 Fluorescence imaging of mitochondrial membrane potential in HeLa cells

<Imaging Filter>
Green : 488 nm (Ex), 500-550 nm (Em), Red : 561 nm (Ex), 560-610 nm (Em)

Mitochondrial membrane potential detection of apoptosis-induced Jurkat cells

1. Jurkat cells(1.0×10^6 cells/mL, 2 mL) in RPMI (10% fetal bovine serum, 1% penicillin-streptomycin) were transfered to a 5 mL tube.
2. The medium was removed and staurosporine in RPMI (0 or 2.5 μg/L, 2 mL) was added to each tube, and then the cells were incubated at 37 °C for 150 minutes in a 5% CO_2 incubator.
3. JC-1 working solution (4 μmol/L, 2 mL) was added, then the cells were incubated at 37 °C for 30 minutes in the 5% CO_2 incubator.
4. The media was removed and the cells were washed with 200 μL of HBSS twice.
5. Imaging Buffer solution (2 mL) was added, and the cells were observed under a fluorescence microscope, and analyzed using a plate reader or a flow cytometer.

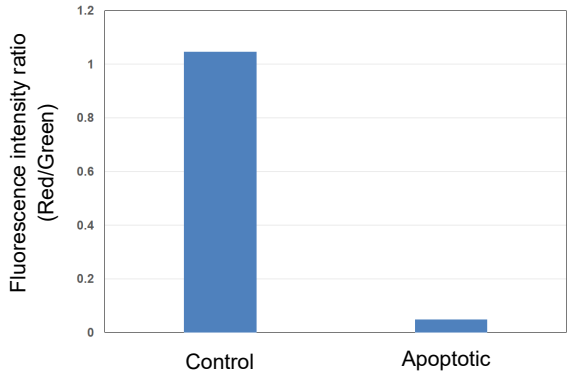
Fluorescence imaging



<Imaging Filter>
Green : 488 nm (Ex), 500-550 nm (Em)
Red : 561 nm (Ex), 560-610 nm (Em)

Figure 3 Fluorescence imaging of mitochondrial membrane potential in Jurkat cells

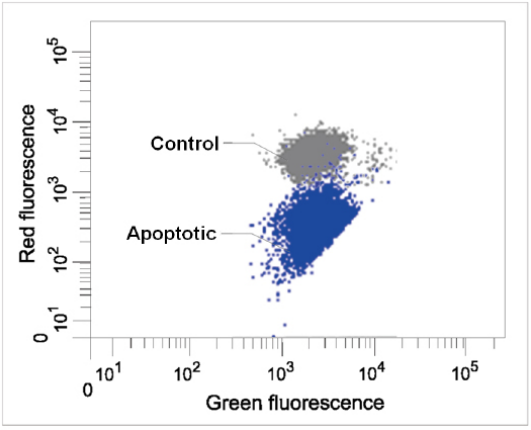
Plate Reader Detection



<Detection Filter>
Green : 485 nm (Ex), 525-545 nm (Em)
Red : 535 nm (Ex), 585-605 nm (Em)

Figure 4 Fluorescence intensity ratio of mitochondrial membrane potential in Jurkat cells

Flow Cytometer Detection



<Detection Filter>
Green : 488 nm (Ex), 515-545 nm (Em)
Red : 488 nm (Ex), 564-604 nm (Em)

Figure 5 Flow cytometric analysis of mitochondrial membrane potential in Jurkat cells

References

1) Ferri, K. F. et al., *J. Exp. Med.*, **2000**, *192*, 1081.
2) Matsuda, N. et al., *J. Cell Biol.*, **2010**, *189*, 211.
3) Wang, J. L. et al., *PNAS*, **2000**, *97*, 7124.

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