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I. INTRODUCTION

Mitochondrial Apoptosis Detection Kit (Cat.No.L00291) provides a simple, fluorescent-based method for distinguishing between healthy and apoptotic cells by detecting the loss of the mitochondrial transmembrane potential ($\Delta\Psi$). Disruption of the mitochondrial transmembrane potential is one of the earliest intracellular events that occur in the induction of apoptosis. The kit utilizes the unique lipophilic cationic dye (5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolylcarbocyanine iodide, commonly known as JC-1) for detection. In healthy cells, the negative charge established by the intact mitochondrial membrane potential allows the lipophilic dye, bearing a delocalized positive charge, to enter the mitochondrial matrix where it accumulates. With the increasing of the concentration of JC-1, it aggregates and become fluorescent red. When the mitochondrial potential collapses in apoptotic cells, JC-1 just exists as monomers and do not accumulate within the mitochondria. When dispersed in this manner, JC-1 remains in the cytoplasm in a green fluorescent monomeric form. The fluorescent signals can be easily detected by fluorescence microscopy using a band-pass filter (detects FITC and rhodamine), or analyzed by flow cytometry using FITC channel for green monomers (Ex/Em =488/530 \pm 30 nm) and PI channel for red aggregates (Ex/Em =488/590 \pm 42 nm).

II. KIT CONTENTS

The Mitochondrial Apoptosis Detection Kit (L00291) employs a lipophilic cation, called JC-1. Each kit contains enough reagents for one hundred apoptosis assays.

Kit Components	100 Assays
JC-1	100 μ l
10xIncubation Buffer	20 ml

III. APPLICATIONS

This kit is designed for detection apoptosis cells by using of mitochondrial transmembrane potential. And the results can be observed by flow cytometers, fluorescence microscopes.

IV. KEY FEATURES

- Easy to perform: simple and rapid procedure to perform.
- Fast: all of the procedure takes about 30 minutes.
- Versatile: directly analyzed by flow cytometers and fluorescence microscope.
- Ready to use: All the reagents for test in live cells.
- Highly competitive price

V. STORAGE

The kit remains stable for at least one year if stored at -20°C. While store 10X Incubation Buffer at 4°C after opened. For JC-1, avoid multiple thawing and freezing cycles and protect from light.

VI. MITOCHONDRIAL APOPTOSIS DETECTION KIT PROTOCOL

Before the experiment, dilute 10X Incubation Buffer to 1X Incubation Buffer with 180 ml of distilled water. Pre-warm the 1xIncubation Buffer to 37°C before use (total 2 ml for each assay).

1. Induce cells apoptosis with required way and set negative control without induction at the same time.
2. Collect cells and wash them twice with PBS by centrifugation at 2000 rpm for five minutes. Then, try to discard PBS.

Note: The quantity of collected cells per sample is about 1×10^6 or less. Otherwise, natural apoptosis will influence the results.

3. Add 1 μ l of JC-1 to 500 μ l of pre-warmed 1X Incubation Buffer. Vortex and mix thoroughly as working solution.

Note: JC-1 is poorly soluble in aqueous solutions. After mixing, the working solution can be centrifuged at 13,000 x g for one minute and carefully transfer the supernatant for removing particles,

4. Suspend cells with 500 μ l of the JC-1 working solution.
5. Incubate the cells at 37°C in a humidified incubator containing 5% CO₂ for 15-20 minutes.
6. Centrifuge the cell suspension at 2000rpm for three minutes at room temperature and discard supernatant.
7. Suspend cells with 500 μ l of 1X Incubation Buffer. Centrifuge the cell suspension at 2000 rpm for three minutes at room temperature and discard supernatant.
8. Repeat step 7.
9. Suspend cells with 500 μ l of the pre-warmed 1X Incubation Buffer.
10. Observe the results by flow cytometer or fluorescence microscopy as described as follow immediately.

A. Quantification by Flow Cytometer

Analyze cells immediately following step 9 by flow cytometer. Mitochondria containing JC-1 aggregates in healthy cells are detectable in the propidium iodide channel (usually FL2), and JC-1 monomers in apoptotic cells are detectable in the FITC channel (usually FL1). Therefore, the cells fluorescing green and red represent apoptotic cells and live non-apoptosis cells, respectively.

B. Detection by Fluorescence Microscopy

1. Place the cell suspension from step 9 on a glass slide and cover the cells with a glass cover slip. For adherent cells, grow the cells on a cover slip and perform the entire procedure directly on the slip in a chamber slide. After incubation, wash the cells with 1X Incubation Buffer and invert the slip on a glass slide to observe.
2. Observe cells immediately under a fluorescence microscope using a band-pass filter (detects fluorescein and rhodamine). JC-1 that has aggregated in the mitochondria of healthy cells fluoresces red. In apoptotic cells, JC-1 cannot accumulate in the mitochondria. It remains as monomers in the cytoplasm, and fluoresces green.

NOTE:

1. Protect the JC-1 reagent from light all the time.
2. For some cells are pH sensitive, it is suggested that washing, incubation and stain all use fetal bovine serum to replace Incubation Buffer.

VII. EXAMPLES

Human P388 cells were stimulated with 4 μ M camptothecin for four hours followed by incubation with the reagent in Incubation Buffer for 15 minutes prior to visualization. After washing, cell fluorescence was measured on a flow cytometry and observed with a microscopy.

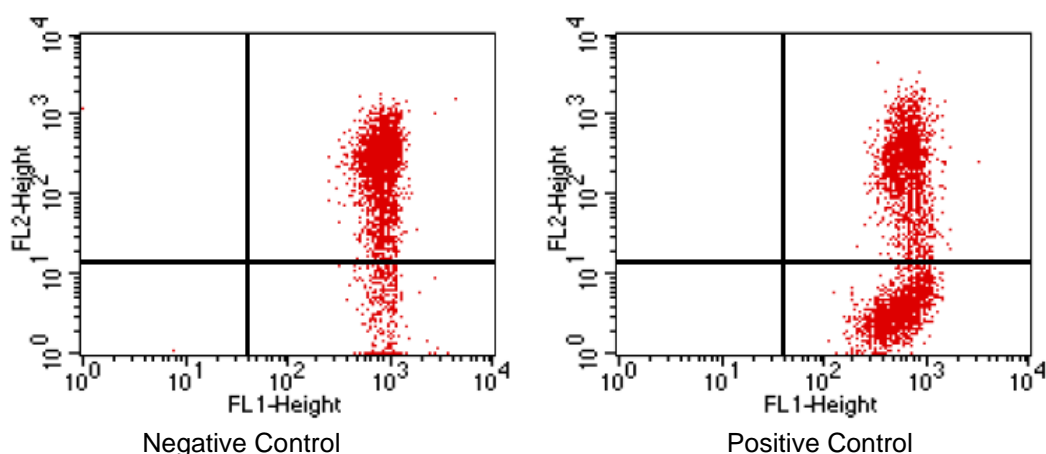
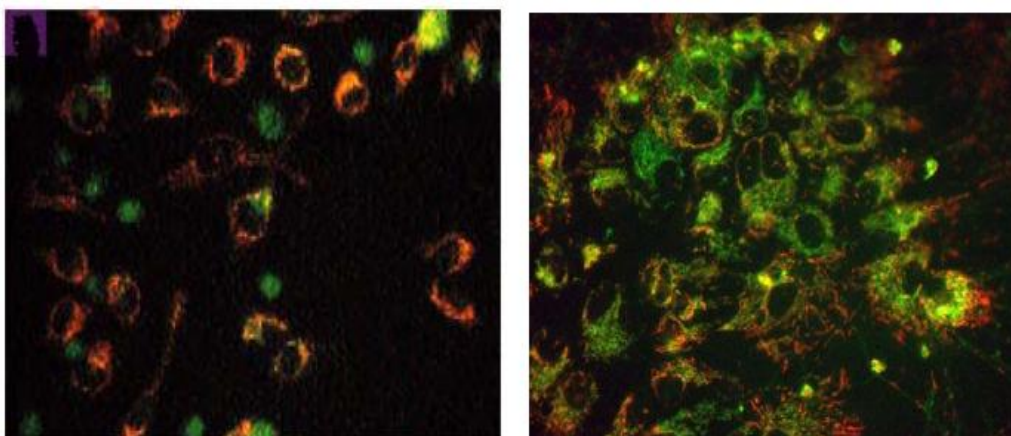


Figure 1 Flow cytometer analyzed apoptotic and healthy cells stained with Mitochondrial Apoptosis Detection Kit (Cat.No.L00291) using FL1 and FL2 channels.



Negative Control

Positive Control

Figure 2 Fluorescence Microscopy analyzed apoptotic and healthy cells stained with Mitochondrial Apoptosis Detection Kit (Cat.No.L00291).

VIII. RELATED PRODUCTS

Annexin V-EGFP Apoptosis Detection Kit:	Cat.No.L00288
Caspase-3 Colorimetric Assay Kit:	Cat.No.L00289
TUNEL Universal Apoptosis Detection Kit (Biotin labeled POD):	Cat.No.L00290
TUNEL Apoptosis Detection Kit (For Adherent Cell, FITC labeled POD):	Cat.No.L00299

IX. ORDERING INFORMATION

Mitochondrial Apoptosis Detection Kit (JC-1):	Cat.No.L00291.
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