Mitochondrial membrane potential assay kit with JC-1

BA14100

Catalog Number:BA14100

Storage: The kit remains stable for at least one year if stored at -20oC. While JC-1 mixed in 10×Incubation Buffer, please store at 4 oC. For JC-1, avoid multiple thawing and freezing cycles and protect from light.

Experimental Methods: Before the experiment, dilute 10×Incubation Buffer to 1×Incubation Buffer with distilled water. Pre-warm the 1×Incubation Buffer to 37oC before use. 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 5 minutes. Then, try to discard PBS. Note: The quantity of collected cells per sample is about 1×106 or less. Otherwise, natural apoptosis will influence the results. 3.Add 1 μl of JC-1 to 500 μl of pre-warmed 1×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.Resuspend cells with 500 μL of the JC-1 working solution. 5.Incubate the cells at 37oC in a humidified incubator containing 5% CO2 for 15-20 minutes. 6.Centrifuge the cell suspesion at 2000 rpm for three minutes at room temperature and discard supernatant. 7.Resuspend cells with 500 μL of 1×Incubation Buffer. Centrifuge the cell suspesion at 2000 rpm for three minutes at room temperature and discard supernatant. 8.Repeat step 7. 9.Resuspend cells with 500 μL of the pre-warmed 1×Incubation Buffer. 10.Observe the results by flow cytometer or fluorescence microscopy as described as following immediately. A. Quantification by Flow Cytometry Analyze cells immediately following step 9 by flow cytometry. 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 1×Incubation Buffer and invert the slip on a glass slide to abserve. 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.

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