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Product Information

Mitochondria Membrane Potential Kit
JC-10 Assay for Microplate Readers

Catalog Number **MAK159**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Mitochondria generate a potential across their membranes due to the activities of enzymes of the electron transport chain. During apoptosis, collapse of the mitochondrial membrane potential (MMP) 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.

This kit utilizes JC-10, a superior alternative to JC-1, for determining the loss of the MMP in cells. Although JC-1 is widely used in many labs, its poor water solubility often results in precipitation in aqueous buffers when used at higher concentrations. At higher concentrations, JC-10 exhibits greater aqueous solubility than JC-1. Similar to JC-1, JC-10 is a cationic, lipophilic dye that is concentrated and forms reversible red-fluorescent JC-10 aggregates (λ_{ex} = 540/λ_{em} = 590 nm) in the mitochondria of cells with a polarized mitochondrial membrane. In apoptotic cells, MMP collapse results in the failure to retain JC-10 in the mitochondria and a return of the dye to its monomeric, green fluorescent form (λ_{ex} = 490/λ_{em} = 525 nm). This kit can be used for monitoring apoptosis and for screening apoptosis inhibitors and activators.

Components

The kit is sufficient for assaying five 96 well plates.

100× JC-10, in DMSO Catalog Number MAK159A	0.25 mL
Assay Buffer A Catalog Number MAK159B	25 mL
Assay Buffer B Catalog Number MAK159C	25 mL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorometric assays.
- Fluorescence multiwell plate reader
- Phosphate Buffered Saline Solution
- Carbonyl cyanide 4-(trifluoromethoxy) phenyl-hydrazone (FCCP, Catalog Number C2920 or equivalent), Carbonyl cyanide 3-chlorophenyl-hydrazone (CCCP, Catalog number C2759 or equivalent), or other suitable positive control for apoptosis.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped under ambient conditions and storage at –20 °C, protected from light, is recommended.

Procedure

Sample Preparation for One Plate

Adherent cells: Plate cells overnight in growth medium at 20,000–80,000 cells/well/90 µL for a 96 well plate or 5,000–20,000 cells/well/25 µL for a 384 well plate.

Non-adherent cells: Centrifuge the cells from the culture medium and resuspend the cell pellets with culture medium in poly-D-lysine-coated plates at 100,000–200,000 cells/well/90 µL for a 96 well plate or 25,000–50,000 cells/well/20 µL for a 384 well plate. Centrifuge the plate at 800 rpm for 2 minutes with brake off prior to the experiment.

Note: Each cell line should be evaluated on an individual basis to determine the optimal cell density.

Assay Reaction

Allow all reagents to come to room temperature before use. Briefly centrifuge vials before opening.

1. Prepare the JC-10 Dye Loading Solution by adding 50 µL of the 100× JC-10 to 5 mL of the Assay Buffer A and mixing well. Aliquot the remaining 100× JC-10 and store at –20 °C. Avoid repeated freeze-thaw cycles.
   Notes: The JC-10 Dye Loading Solution is enough for one plate. The amount of JC-10 Dye Loading Solution can be scaled if necessary.

2. Treat cells with 10 µL (96 well plate) or 5 µL (384 well plate) of 10× test compounds for desired period to induce apoptosis. In parallel, set up negative (vehicle only) and positive (FCCP, CCCP, or other suitable positive control) control samples. Incubate the cells in a 5% CO_2, 37 °C incubator for desired amount of time sufficient to induce apoptosis.
   Note: It is not necessary to wash cells before adding test compounds. However, if test compounds are serum sensitive, samples can be grown in serum-free medium or growth medium and serum factors can be removed before adding compounds at 1× in PBS.

3. Add 50 µL/well for 96 well plate and 12.5 µL/well for 384 well plate of the JC-10 Dye Loading Solution into each of the sample wells. Incubate the cells, protected from light, in a 5% CO_2, 37 °C incubator for 30–60 minutes.
   Note: The appropriate incubation time depends on the individual cell type and cell concentration used.

4. Add 50 µL/well for 96 well plate or 12.5 µL/well for 384 well plate of Assay Buffer B to each of the sample wells. Do not wash the cells after loading. For non-adherent cells, it is recommended to centrifuge cell plates at 800 rpm for 2 minutes with the brake off after adding Assay Buffer B.

5. Monitor the fluorescence intensity (λ_{ex} = 490/ λ_{em} = 525 nm) and (λ_{ex} = 540/λ_{em} = 590 nm) for ratio analysis. The ratio of red/green fluorescence intensity is used to determine MMP.

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