3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Mitochondria Membrane Potential Kit
JC-10 Assay for Flow Cytometry

Catalog Number **MAK160**
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 100 assays.

200× JC-10 in DMSO Catalog Number MAK160A	0.25 mL
Assay Buffer Catalog Number MAK160B	50 mL

Reagents and Equipment Required but Not Provided.

- Carbonyl cyanide 4-(trifluoromethoxy) phenyl-hydrazone (FCCP, Catalog Number C2920 or equivalent) or Carbonyl cyanide 3-chlorophenyl-hydrazone (CCCP, Catalog number C2759 or equivalent)

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

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

1. For each sample, prepare cells in warm medium or buffer at a density between 5×10^5 and 1×10^6 cells/mL.
   Note: Each cell line should be evaluated on an individual basis to determine the optimal cell density.

2. Treat cells with test compounds for desired period to induce apoptosis. In parallel, set up negative (vehicle only) and positive (FCCP or CCCP at 2–10 μM) control samples. Incubate the cells in a 5% CO_2, 37 °C incubator.
   Note: FCCP or CCCP treatment for 15–30 minutes is sufficient to induce apoptosis in most cell lines. The concentration of FCCP or CCCP necessary to induce apoptosis may need to be titrated.

3. Prepare the JC-10 Dye Loading Solution by adding 25 µL of the 200× JC-10 to 5 mL of Assay Buffer and mixing well.

4. Transfer 2–5 × 10^5 cells to a tube. Centrifuge and solution from cells.
   Note: If working with adherent cells, add 0.5 mM EDTA to gently remove cells from plate.
   Wash 1 time with serum-containing medium prior to the incubation with the JC-10 Dye Loading Solution.

5. Resuspend cells in 500 µL of the JC-10 Dye Loading Solution to each of the samples. Incubate the cells in a 5% CO_2, 37 °C incubator for 15–60 minutes, protected from light.
   Note: The appropriate incubation time depends on the individual cell type and cell concentration used.

6. Centrifuge the cells at 1,000 rpm for 4 minutes. Resuspend the cells in 1 mL of Assay Buffer or buffer of choice.

7. Monitor the fluorescence intensity using a flow cytometer in the FL1 (green) and FL1 (red) channels.

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