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Product Information

Mitochondria Membrane Potential Kit
for Flow Cytometry, Near Infrared Fluorescence

Catalog Number **MAK148**
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 is optimized for the analysis of the MMP by flow cytometry on instruments with a 635 nm laser. The cationic hydrophobic mitochondrial potential dye accumulates in normal mitochondria, most likely due to the mitochondrial potential, resulting in an increase in fluorescence (λ_{ex} = 635 nm/λ_{em} = 660 nm). In apoptotic cells, MMP collapse results in decreased fluorescence. This kit can be used for monitoring apoptosis and for screening apoptosis inhibitors and activators.

Components

The kit is sufficient for 100 assays.

200× Mitochondrial Potential Dye Catalog Number MAK148A	0.5 mL
Assay Buffer Catalog Number MAK148B	100 mL

Reagents 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 Material 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 1 mL of 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 5–50 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. Add 5 µL of the 200× Mitochondrial Potential Dye to each of the samples. Incubate the cells in a 5% CO_2, 37 °C incubator for 15–30 minutes.
   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 200× Mitochondrial Potential Dye.
4. Centrifuge the cells at 1,000 rpm for 4 minutes. Resuspend the cells in 1 mL of Assay Buffer or buffer of choice.
5. Monitor the fluorescence intensity using a flow cytometer equipped with a 635 nm red diode laser and a 661 nm filter (λ_{ex} = 635 nm/λ_{em} = 660 nm).

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