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# Product Information

**Mitochondria Membrane Potential Kit**
for Microplate Readers

Catalog Number **MAK147**
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 detection of the loss of the MMP in cells. The cationic hydrophobic mitochondrial potential dye accumulates in normal mitochondria, most likely due to the mitochondrial potential, resulting in an increase in fluorescence ($\lambda_{ex}$ = 540/$\lambda_{em}$ = 590 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 assaying five 96 well plates.

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|---|---|
| 200× Mitochondrial Potential Dye<br>Catalog Number MAK147A | 0.25 mL |
| Assay Buffer A<br>Catalog Number MAK147B | 50 mL |
| Assay Buffer B<br>Catalog Number MAK147C | 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
- 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

Sample Preparation for One Plate

Adherent cells: Plate cells overnight in growth medium at 20,000–80,000 cells/well/100 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/100 µL for a 96 well plate or 25,000–50,000 cells/well/25 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 Dye Loading Solution by adding 50 µL of the 200× Mitochondrial Potential Dye to 10 mL of the Assay Buffer A and mixing well. Aliquot remaining 200× Mitochondrial Potential Dye and store at –20 °C. Avoid repeated freeze-thaw cycles.
   Notes: The Dye Loading Solution is enough for one plate. It is best used within 2 hours. The amount of dye loading solution can be scaled if necessary.

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. Remove the cell medium. It is critical to remove the cell medium before adding the Dye Loading Solution.

4. Add 100 µL/well for 96 well plate and 25 µL/well for 384 well plate of the Dye Loading Solution into each of the sample and control wells. Incubate the cells in a 5% $CO_2$, 37 °C incubator for 15–30 minutes.
   Note: The appropriate incubation time depends on the individual cell type and cell concentration used.

5. 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 and control 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.

6. Incubate the plate for 10–30 minutes.

7. Monitor the fluorescence intensity ($\lambda_{ex}$ = 540/ $\lambda_{em}$ = 590 nm).

**Results**
In live, non-apoptotic cells, the orange fluorescence intensity is increased as the mitochondrial potential dye is accumulated in the mitochondria. In apoptotic and dead cells, stain intensity is decreased due to the collapse of the MMP.

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