IMMUNOLOGICAL SCIENCES Product Data Sheet

# IKA403 MitoBright Deep Red Probe Assay Kit

**Size: 100 Assays**

| Cat. | Products | 100 Assays | Storage |
|---|---|---|---|
| IKA403A | MitoBright Deep Red Probe Powder | 1.5 μg × 5 | -20°C, shading light |
| IKA403B | MitoBright Deep Red Probe Solvent | 200 μL | -20°C, shading light |
| | Manual | One Copy | |

## Storage

MitoBright Deep Red Probe Powder and MitoBright Deep Red Probe Solvent can be stored at -20°C with shading light for 1 year.

## Detection Principle

MitoBright Deep Red Probe Assay Kit is an assay kit that labels mitochondria of living cells and excites Deep Red fluorescence. MitoBright Deep Red Probe is a lipophilic, deep red fluorescent probe with cell membrane permeability, which can passively diffuse through the cell membrane of living cells and enrich in mitochondria, exhibiting membrane potential dependence. .The stained cells can be fixed (aldehyde fixative) or permeabilized (aldehyde decontaminant such as Triton X-100) according to the needs of the subsequent experiments, and there will be a slight decrease in fluorescence intensity after fixation.

## Detection Sample Types

☑ Adherent Cells

☑ Suspension Cells

## Materials Not Supplied

1) **Reagents**

   75% ethanol, cell culture medium, sterile PBS buffer and paraformaldehyde fixative

2) **Instruments**

   Centrifuge, $CO_2$ incubator, fluorescence microscope, flow cytometer, biosafety cabinet

3) **Consumable Materials**

   Petri dishes, pipette, 24-well plates, cell crawlers, microscope slides

## Experimental Protocol

### ➢ Reagent preparation

**Preparation of MitoBright Deep Red Probe Preservation Solution (200 μM):** Take out the MitoBright Deep Red Probe Powder, centrifuge at 12000 rpm for 1 min, make the powder gather at the bottom of the tube, add 13.8 μL of MitoBright Deep Red Probe Solvent to 1.5 μg of the powder per tube, gently mix fully and aliquot into smaller quantities for -20 °C storage with shading light.

### ➢ For fluorescence microscope

a) Carefully aspirate and discard the medium from the adherent cells, add 1 mL of PBS buffer per well to infiltrate and wash the cells for 3 min, and remove the PBS buffer.

b) Preparation of MitoBright Deep Red Probe Staining Solution (200 nM): dilute 200 μM MitoBright Deep Red Probe Preservation Solution to 200 nM MitoBright Deep Red Probe Staining Working Solution with basal medium (without serum). Please refer to the table below (100 μL MitoBright Deep Red Probe Staining Working Solution per well for 96-well plates or 500 μL per well for 24-well plates).

| Component | MitoBright Deep Red Probe Staining Working Solution (200 nM) | | |
|---|---|---|---|
| **MitoBright Deep Red Probe Preservation Solution (200 μM)** | 0.5 μL | 1 μL | 2 μL |
| **Basal medium** | 500 μL | 1000 μL | 2000 μL |

Note: A negative control is recommended for each experiment; the negative control is the cells resuspended in basal medium and without MitoBright Deep Red Probe.

c) Add MitoBright Deep Red Probe Staining Working Solution (200 nM) at the ratio of 500 μL per well in a 24-well plate, and incubate for 30 min at 37°C with shading light.

d) Carefully aspirate the staining working solution, add 1 mL of PBS buffer to each well, wash the cells for 3~5 min, remove the PBS buffer, add 500 μL of PBS buffer to infiltrate the cells.

e) Observed and photographed directly under an inverted fluorescence microscope with Cy5 filter set. (MitoBright Deep Red Probe is Deep Red fluorescent, Ex/Em= 644 nm/665 nm).

f) If the adherent cells are cultured on the glass crawler in advance, the cell crawler can be removed after staining, placed on a slide, and then observed and photographed using a fluorescence microscope.

Note: Keep the sample moist during the experiment to prevent the failure of the experiment caused by dry slides.

g) For suspended cells, resuspend the cells at the ratio of 1~5×$10^5$ cells with 500 μL of 200 nM MitoBright Deep Red Probe Staining Solution , incubate at 37°C for 30 min with shading light, add 1 mL of PBS buffer, and centrifuge at 300×g for 5 min to wash the cells, then discard the supernatant, take 10~20 μL of PBS buffer to resuspend the cell precipitate, drop the cell suspension on the slide, and gently cover the coverslip to observe and take pictures under the microscope.

Note:

a) It is recommended to use freshly prepared the staining working solution be dispensed and use out in the same day.

b) When taking pictures with fluorescence microscope, its light intensity is too strong which will cause fluorescence quenching, so the light intensity can be appropriately reduced, or fixed with paraformaldehyde at room temperature and protected from light for 20 min, and then washed with PBS, and then observed and photographed using a fluorescence microscope. The fixed samples were stored at 4°C and protected from light for 3 days after infiltration with PBS, and the fluorescence brightness was stable and unchanged.

- **For flow cytometry**

a) Collect the cells and centrifuge at 300×g for 5 min at room temperature, discard the supernatant, resuspend the cell pellet with 1 mL of basal medium, then centrifuge at 300×g for 5 min at room temperature and keep the cell pellet.

b) Preparation of MitoBright Deep Red Probe Staining Solution (5 nM): Due to the high sensitivity of the flow cytometry instrument, MitoBright Deep Red Probe Staining Solution (200 nM) needs to be further diluted, refer to the following table to dilute MitoBright Green Probe Staining Solution (200 nM) to 5 nM (ready to use). According to the amount of single experiment, 500 μL of 5 nM MitoBright Deep Red Probe Staining Working Solution for 1~5×$10^5$ cells, refer to the following table to prepare sufficient amount of MitoBright Deep Red Probe Staining Working Solution (5 nM):

| Component | MitoBright Deep Red Probe staining working solution (5 nM) | | | |
|---|---|---|---|---|
| **MitoBright Deep Red Probe Preservation Solution (200 nM)** | 12.5 μL | 25 μL | 50 μL | 100 μL |
| **Basal medium** | 487.5 μL | 975 μL | 1950 μL | 3900 μL |

Note: A negative control is recommended for each experiment; the negative control is the cells resuspended in basal culture medium without MitoBright Deep Red Probe.

c) Take 1~5×$10^5$ cells per group, add 500 µL of MitoBright Deep Red Probe Staining Working Solution (5 nM) to resuspend the cell pellet, gently mix fully, incubate at 37°C in the incubator for 15~20 min with shading light.

d) Add 1 mL of PBS buffer to each group, gently mix fully, centrifuge at 300×g for 5 min at room temperature, and discard the supernatant.

e) Resuspend the cells with 1 mL of PBS buffer, gently mix fully, centrifuge at 300×g for 5 min at room temperature, and discard the supernatant.

f) Resuspend the cell pellet with 100~200 µL PBS buffer and analyzed by flow cytometry in the APC channel.

Note:

a) When co-staining with other antibodies and other reagents, the stained cells can be fixed with 4% formaldehyde or paraformaldehyde for 30 min at room temperature and protected from light, and then washed by centrifugation before detection.

b) The brightness of mitochondrial fluorescence will be decreases after fixation. To maintain optimal detection resolution, the concentration of MitoBright Deep Red Probe staining solution can be increased to 5-10 nM for samples that require fixation.

## Typical Results

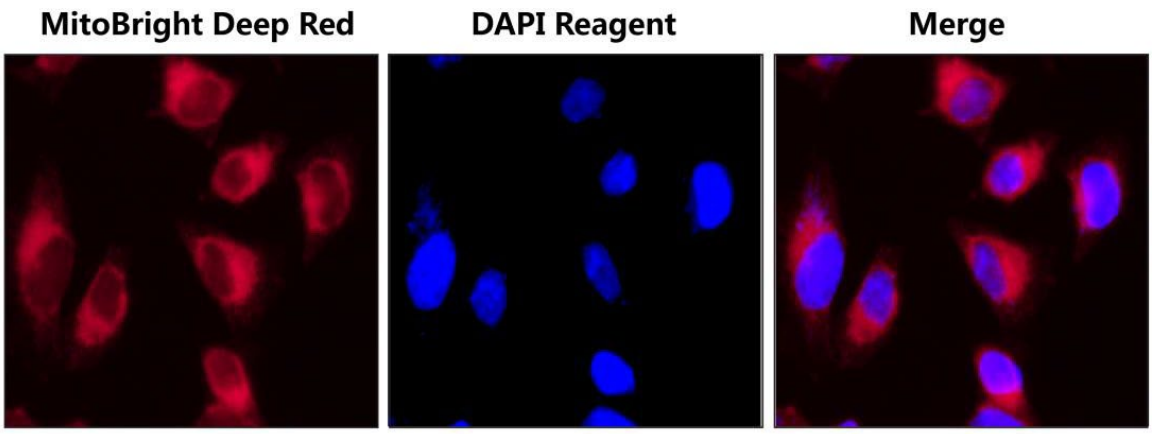


**Figure 1.** HELA cells were co-stained with MitoBright Deep Red Probe and DAPI Reagent (25 µg/mL)

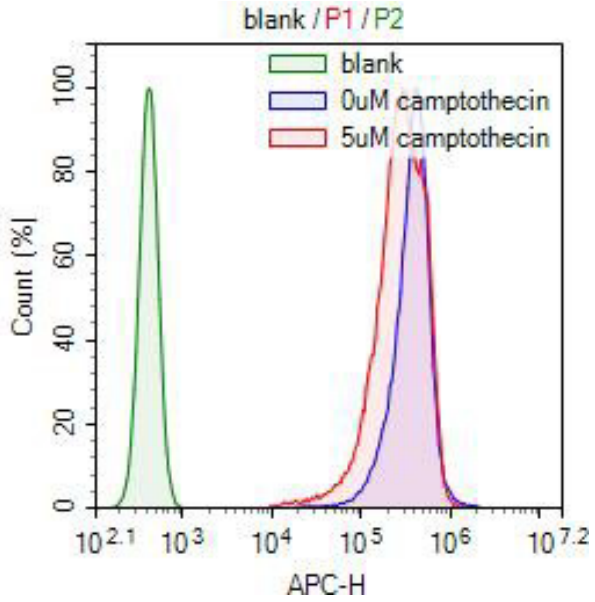


**Figure 2 (left).** Jurkat cells were induced with 0μM or 5 μM camptothecin for 4h, and then the cells were collected and stained with MitoBright Deep Red Probe. The results showes that the mitochondrial membrane potential decreased with enhanced apoptosis, and the MitoBright Deep Red Probe fluorescence brightness decreased.

## Cautions

1. This product is for research use only.
2. For your safety and health, please wear laboratory overalls and disposable gloves for operation, and follow the laboratory reagent operating procedures.
3. This product is used for intact mitochondrial labelling in living cells, and cannot be used to stain cells after fixation, but cells can be fixed after probe staining with some degree of decrease in fluorescence intensity present.
4. The dry powder state of MitoBright Deep Red Probe Powder in this product is more stable. After adding MitoBright Deep Red Probe Solvent to dissolve it, it is recommended to aliquot to smaller quantities and use out within 6 months, and avoid repeated freezing and thawing.