MitoBright Red Probe Assay Kit

Cat. No: E-CK-A402 **Size: 20Assays/100Assays**

Cat.	Products	20 Assays	100 Assays	Storage
E-CK-A402A	MitoBright Red Probe Powder	1.5 μg	1.5 μg × 5	-20°C, shading light
E-CK-A402B	MitoBright Red Probe Solvent	20 μL	100 μL	-20°C, shading light
	Manual		One Copy	

Storage

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

Detection Principle

Elabscience® MitoBright Red Probe Assay Kit is an assay kit that labels mitochondria of living cells and excites red fluorescence. MitoBright Red Probe is a lipophilic cationic fluorescent dye with a mild sulfhydryl-reactive chloromethyl functional group that covalently reacts with sulfhydryl groups on proteins and peptides in the mitochondrial matrix to form thioester bonds, which can be aggregated within the mitochondria. The red probe is stable in staining. The stained cells can be fixed (aldehyde fixative) or permeabilised (aldehyde decontaminant such as Triton X-100) according to the needs of the subsequent experiments, and its probe still exists in the mitochondria after 1~2 weeks storage at 4°C, but the fluorescence intensity will be decreased to a certain extent. When the membrane potential of mitochondria decreases, the fluorescence brightness of MitoBright Red Probe decreases to some extent.

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) **Materials**

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

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Experimental Protocol

➢ Reagent preparation

Preparation of MitoBright Red Probe Preservation Solution (200 μM): Take out the MitoBright Red Probe Powder, centrifuge at 12000 rpm for 1 min, make the powder gather at the bottom of the tube, add 14.1 μL of MitoBright 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 Red Probe Staining Solution (200 nM): dilute 200 μM MitoBright Red Probe Preservation Solution to 200 nM MitoBright Red Probe Staining Working Solution with basal medium (without serum). Please refer to the table below (100 μL MitoBright Red Probe Staining Working Solution per well for 96-well plates or 500 μL per well for 24-well plates).

Component	MitoBright Red Probe Staining Working Solution（200 nM）		
MitoBright 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 without MitoBright Red Probe.

c) Add MitoBright 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 TRITC filter set. (MitoBright Red Probe is Red fluorescent, Ex/Em= 579 nm/599 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

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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 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 Red Probe Staining Solution (80 nM): Due to the high sensitivity of the flow cytometry instrument, MitoBright Red Probe Staining Solution needs to be diluted to 80 nM (ready to use). According to the dosage of a single experiment, 500 μL of 80 nM MitoBright Red Probe Staining Solution for 1~5×10^5 cells, and refer to the table below to prepare the sufficient amount of staining working solution (80 nM):

Component	**MitoBright Red Probe staining working solution (80 nM)**			
MitoBright Red Probe Preservation Solution (200 μM)	0.2μL	0.4μL	0.8 μL	2 μL
Basal medium	500 μL	1000 μL	2000 μL	5000 μL

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

c) Take 1~5×10^5 cells per group, add 500 μL of MitoBright Red Probe Staining Working Solution (80 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 Percp/Cy5.5 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 Red Probe staining solution can be increased to 80-120 nM for samples that require fixation.

Typical Results

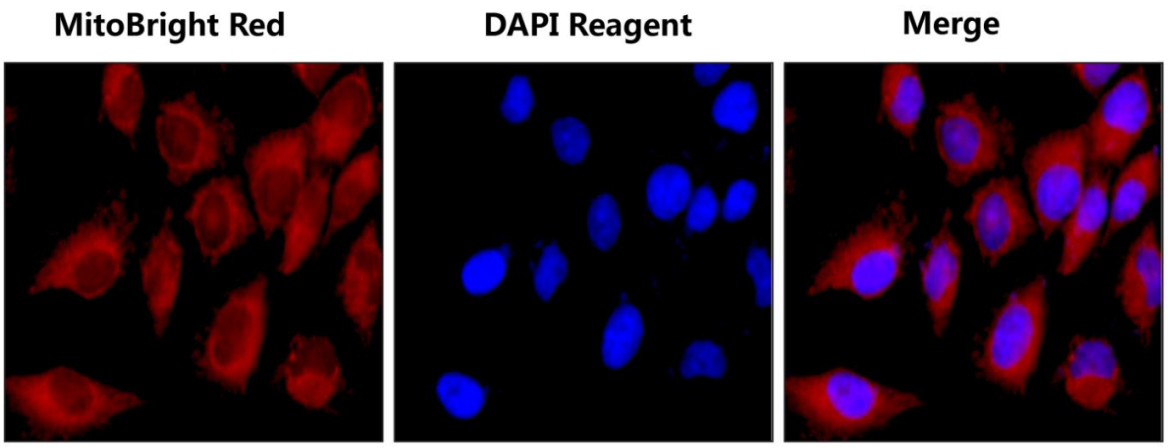

Figure 1. HELA cells were co-stained with MitoBright Red Probe and DAPI Reagent (25 μg/mL) (E-CK-A163).

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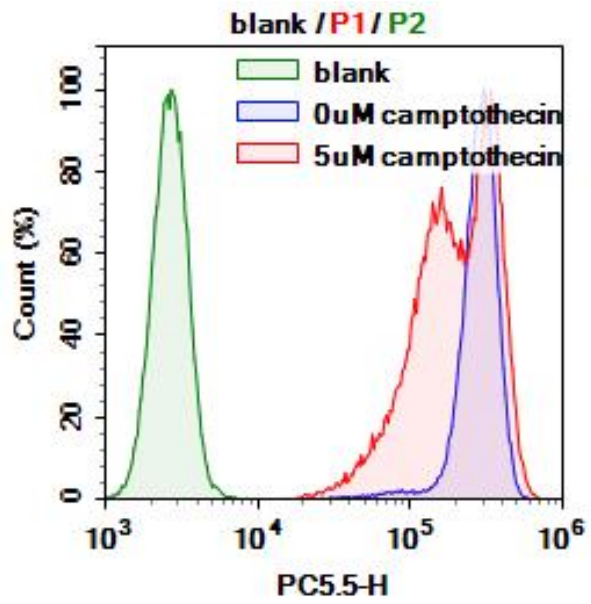

Figure 2 (left). Jurkat cells were induced with 0μM or 5 μM camptothecin for 4h, and then the cells were collected and stained with MitoBright Red Probe. The result shows that the mitochondrial membrane potential decreased with enhanced apoptosis, and the MitoBright Red Probe fluorescence brightness 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 Red Probe Powder in this product is more stable. After adding MitoBright 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.

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