IMMUNOLOGICAL SCIENCES Product Data Sheet

IKA401 MitoBright Green Probe Assay Kit

Size: 100 Assays

Cat.	Products	100 Assays	Storage
IKA401A	MitoBright Green Probe Powder	3 μg ×5	-20°C, shading light
IKA401B	MitoBright Green Probe Solvent	100 μL	-20°C, shading light
	Manual	One Copy	

Storage

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

Detection Principle

MitoBright Green Probe Assay Kit can be used to label mitochondria in living cells to elicit green fluorescence. MitoBright Green Probe is a derivative of acridine orange, which has cell membrane permeability and can specifically bind to cardiolipin on the mitochondrial membrane. It can be used to qualitatively or quantitatively determine the mitochondrial mass as well as the toxicity and damaging effects of free radicals, oxides, etc., on the cellular mitochondria by analyzing the changes in the cardiolipin of the mitochondrial membrane. The probe stains stably and the staining results are not affected by changes in mitochondrial membrane potential. 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 the fluorescence intensity will be slightly decreased 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, inverted 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 Green Probe Preservation Solution (500 μM): Take out the MitoBright Green Probe Powder, centrifuge at 12000 rpm for 1 min, make the powder gather at the bottom of the tube, add 12.7 μL of MitoBright Green Probe Solvent to 3 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 Green Probe Staining Solution (500 nM): dilute 500 μM MitoBright Green Probe Preservation Solution to 500 nM MitoBright Green Probe Staining Solution with basal medium (without serum). Please refer to the table below (100 μL MitoBright Green Probe Staining Solution per well for 96-well plates or 500 μL per well for 24-well plates).

Component	**MitoBright Green Probe Staining Working Solution（500 nM）**		
MitoBright Green Probe Preservation Solution (500 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 Green Probe.

c) Add MitoBright Green Probe Staining Working Solution (500 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 FITC filter set. (MitoBright Green Probe is green fluorescent, Ex/Em= 495 nm/519 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 500 nM MitoBright Green 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 Green Probe Staining Solution (5 nM): Due to the high sensitivity of the flow cytometry instrument, MitoBright Green Probe Staining Solution (500 nM) needs to be further diluted, refer to the following table to dilute MitoBright Green Probe Staining Solution (500 nM) to 5 nM (ready to use). According to the amount of single experiment, 500 μL of 5 nM MitoBright Green Probe Staining Solution for 1~5×10^5 cells, refer to the following table to prepare sufficient amount of MitoBright Green Probe Staining Working Solution (5 nM):

Component	**MitoBright Green Probe staining working solution (5 nM)**			
MitoBright Green Probe Staining Working Solution (500 nM)	5 μL	10 μL	20 μL	50 μL
Basal medium	495 μL	990 μL	1980 μL	4950 μL

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

c) Take 1~5×10^5 cells per group, add 500 μL of MitoBright Green 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 FITC 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 Green Probe staining solution can be increased to 5-10 nM for samples that require fixation.

Typical Results

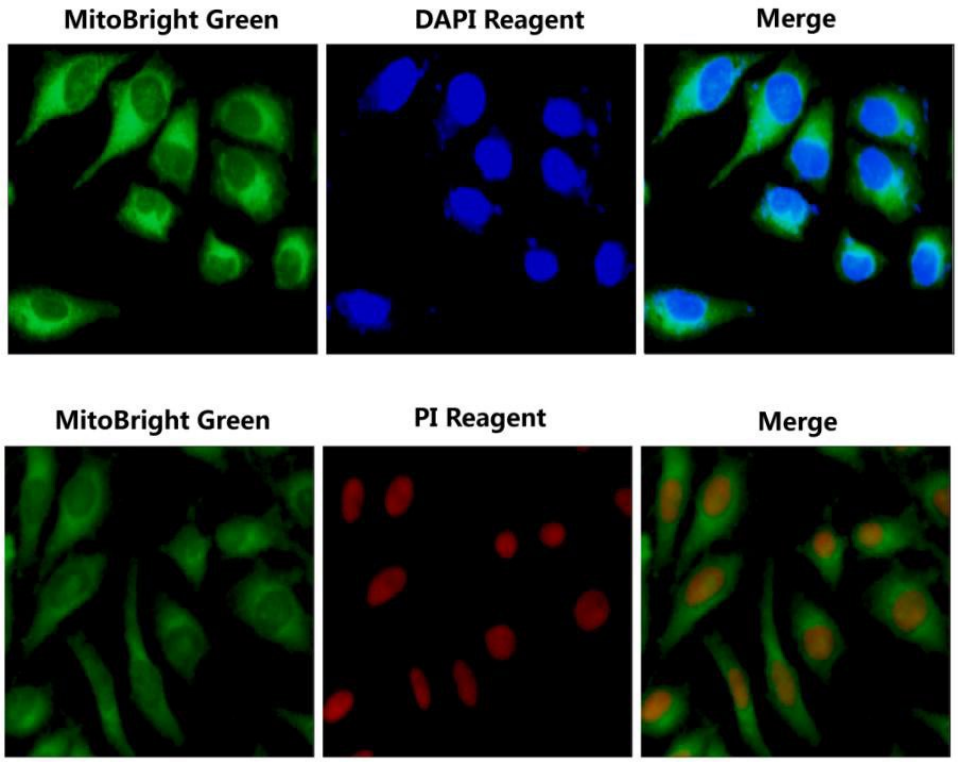

Figure 1. HELA cells were co-stained with MitoBright Green Probe and DAPI Reagent (25 μg/mL) or PI Reagent (750 μM).

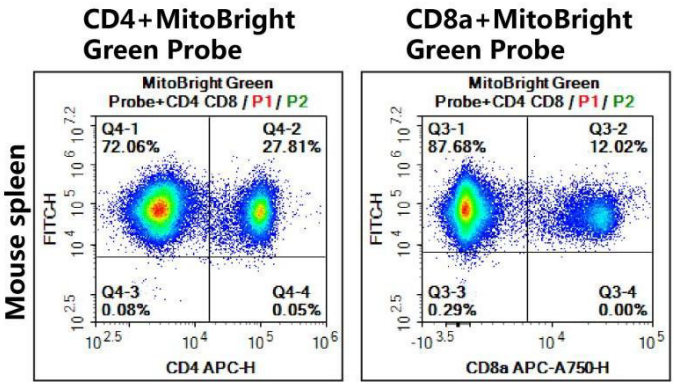

Figure 2 (left). Mouse spleen cells were co-stained with MitoBright Green Probe, APC Anti-Mouse CD4 Antibody [GK1.5] and Alexa Fluor Red 780 Anti-Mouse CD8a Antibody [53-6.7]

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 Green Probe Powder in this product is more stable. After adding MitoBright Green Probe Solvent to dissolve it, it is recommended to aliquot to smaller quantities and use out within 6 months, and the number of repeated freezing and thawing should not be more than 5 times.