TraKine™ Mitochondrion Staining Kit (Orange Fluorescence)

Cat #: KTC4004 Size: 100 T/500 T/2000 T

	Mitochondrion Staining Kit (Orange Fluorescence)		
REF	**Cat #:** KTC4004	LOT	**Lot #:** Refer to product label
	Fluorescence excitation/emission: Orange fluorescent probe (Ex/Em=579/599 nm)		
	Storage: Stored at -20℃ for 6 months		

Assay Principle

Mitochondria is a double-membrane-bound organelle found in most eukaryotic cells. The function of mitochondria is to provide cellular energy. Moreover, mitochondria are involved in other tasks, such as signaling, cellular differentiation, and cell death, as well as maintaining control of the cell cycle and cell growth. Mitochondria have been implicated in several human diseases, including mitochondrial disorders, cardiacdysfunction, heart failure and autism. Mitochondria may play an important role in these cellular processes. TraKine™ Mitochondrion Staining Kit (Orange Fluorescence) is a set of fluorescence imaging tools to label mitochondria of live cells. This kit uses a proprietary mitochondrial orange fluorescent probe (Ex/Em=579/599 nm), which, like other probes, the kit uses a proprietary dye that selectively accumulates in mitochondria probably vial the mitochondrial membrane potential gradient. MitoOrange, a hydrophobic compound, easily permeates intact living cells and can be retained after formaldehyde fixation and permeabilization. The dye is suitable for double labeling experiments, and its orange fluorescent probes is well distinguished from other green fluorescent probes. The fluorescence can be measured using fluorescence imaging, high-content imaging, microplate fluorometry, or flow cytometry. This kit is suitable for proliferating and non-proliferating cells, and can be used for both suspension and adherent cells.

Materials Supplied and Storage Conditions

Kit components	Size			Storage conditions
	100 T	500 T	2000 T	
MitoOrange™ (1000×)	50 µL	250 µL	1 mL	-20℃, protected from light
Assay Buffer (10×)	5 mL	25 mL	100 mL	4℃

Materials Required but Not Supplied

- Fluorescence Microscope or Flow Cytometer
- 24 well dish (cell culture)
- Microcentrifuge
- Phosphate buffered saline (PBS) (pH 7.4)

Reagent Preparation

MitoOrange™ (1000×)： Before use, warm to room temperature. The remaining working solution can be stored at -20℃ after aliquoting to avoid repeated freezing and thawing.

Assay Buffer (1×)： Before use, dilute to 1×Assay Buffer with deionized water, and then heat to 37℃. Store at 4℃.

Staining Solution：Add 1 μL MitoOrange™ (1000×) to 1 mL 1×Assay Buffer, and increase the volume according to the number of experiments.

Assay Procedure

Note: As the optimal staining conditions may vary among different cell types, we recommend that a suitable concentration of MitoOrange™ should be determined individually.

A. Quantification by Flow Cytometry

Note: We recommend keeping unstained control cells (without MitoOrange™) suspended in 1×Assay Buffer to set up the flow cytometer instrument.

1. For non-adherent cells, Collect 1-5×10^5 cells by centrifugation (4℃, 300 g, 5 min). Wash with PBS twice and discard the PBS. For adherent cells, using Trypsin (EDTA free) to digest cells firstly and then centrifugation.
2. Resuspend the cells pellet in 0.5 mL Staining Solution.
3. Incubate the cells at 37℃ for 15-45 min in the dark.
4. Centrifuge cells at 500 g and discard supernatant.
5. Wash cell pellet with PBS twice.
6. Resuspend cell pellet in 0.5 mL of the pre-warmed PBS and analyze the cells by flow cytometry using PI channel (usually FL2).

B. Detection by Fluorescence Microscope

1. For suspension cells: Follow the protocol for flow cytometry from step1 to step 6 and place the cell suspension on a glass slide. Cover the cells with a glass coverslip. Analyze cells by fluorescence microscopy.
2. For adherent cells: the suggested protocol is as below.

2.1 Grow cells directly on a coverslip in 24 well dish. Incubate in a CO_2. Incubator at 37℃ for at least 24 h before treatment.

2.2 Wash cells with PBS twice.

2.3 Add 0.5 mL of Staining solution to cells and incubate at 37℃ for 30 min in the dark.

2.4 Wash cells with pre-warm PBS twice.

2.5 Invert coverslip on a glass slide and visualize cells fluorescence microscopy (Ex/Em=579/599 nm).

Recommended Products

Catalog No.	Product Name
KTC4001	TraKine™ Cell Plasma Membrane Staining Kit (Green Fluorescence)
KTC4002	TraKine™ Cell Plasma Membrane Staining Kit (Orange Fluorescence)
KTC4003	TraKine™ Mitochondrion Staining Kit (Green Fluorescence)
KTC4005	TraKine™ Mitochondrion and Nuclear Staining Kit
KTC4008	TraKine™ F-actin Staining Kit (Green Fluorescence)
KTC4009	TraKine™ F-actin Staining Kit (Orange Fluorescence)

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.