Customer service: service@abbkine.com
Technical support: support@abbkine.com
Website: https://www.abbkine.com

TraKine™ Mitochondrion and Nuclear Staining Kit

Cat #: KTC4005 Size: 100 T/500 T/2000 T

	Mitochondrion and Nuclear Staining Kit		
REF	**Cat #:** KTC4005	LOT	**Lot #:** Refer to product label
	Fluorescence excitation/emission: Orange fluorescent MitoOrange™ (Ex/Em=579/599 nm) and blue-fluorescent Hoechst 33342 (Ex/Em=350/461 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 and Nuclear Staining Kit provides two stains--Orange fluorescent MitoOrange™ (Ex/Em=579/599 nm) and blue-fluorescent Hoechst 33342 (Ex/Em=350/461 nm, when bound to DNA)-for highly selective mitochondrial and nuclear staining, respectively, in live, GFP-transfected cells. Both dyes are retained after formaldehyde fixation and permeabilization. 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
Hoechst 33342 (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
- Pipettes and pipette tips, Deionized water
- 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.

Hoechst 33342 (1×)：Before use, warm to room temperature. Add 1 μL Hoechst 33342 (1000×) to 1 mL 1×Assay Buffer, and increase the volume according to the number of experiments. The remaining Hoechst 33342 (1000×) can be stored at -20℃ after aliquoting to avoid repeated freezing and thawing.

Assay Procedure

Note: As the optimal staining conditions may vary among different cell types, we recommend that a suitable concentration of MitoOrange™ and Hoechst 33342 should be determined individually.

A. Quantification by Flow Cytometry

Note: We recommend keeping unstained control cells (without MitoOrange™ and Hoechst 33342) 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 the cells pellet in 0.5 mL Hoechst 33342 (1×), mix well.
7. Incubate the cells at room temperature for 3-5 min in the dark.
8. Centrifuge cells at 500 g and discard supernatant.
9. Wash cell pellet with PBS twice.
10. Resuspend cell pellet in 0.5 mL of the pre-warmed PBS and analyze the cells by flow cytometry.

B. Detection by Fluorescence Microscope

1. For suspension cells: Follow the protocol for flow cytometry from step A.1 to step A.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 Add 0.5 mL of Hoechst 33342 (1×) to cells and incubate at room temperature for 3-5 min in the dark.
2.6 Wash cells with pre-warm PBS twice.
2.7 Invert coverslip on a glass slide and visualize cells fluorescence microscopy.

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)
KTC4004	TraKine™ Mitochondrion Staining Kit (Orange Fluorescence)

Disclaimer

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