TraKine™ Mitochondrion Staining Kit (Orange Fluorescence)

Item NO.	Product Name
KTC4004	TraKine™ Mitochondrion Staining Kit (Orange Fluorescence)

ATTENTION

For laboratory research use only. Not for clinical or diagnostic use

Version 201809
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INTRODUCTION

Background & 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.

Abbkine Mitochondrion Staining Kits are 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.

Storage/Stability

Refer to list of materials supplied for storage conditions of individual components. Stable for at least 6 months at recommended temperature from date of shipment. Gel pack with blue ice.

Assay Restrictions

- Assay kit is intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

PRODUCT INFORMATION

Materials supplied and Storage conditions

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

Other supplies required, Not Supplied

- Microcentrifuge
- Pipettes and pipette tips
- Fluorescence Microscopy or Flow Cytometer
- 24-well plate for cell culture
- Phosphate-buffered saline (PBS)

Technical hints

- To avoid cross-contamination, change pipette tips between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

ASSAY PROTOCOL

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

Reagent Preparation

MitoOrange™ (1000×): Warm to room temperature before using. Aliquot and store unused MitoOrange™ (1000×) stock solutions at -20°C. Protect from light and avoid repeated freeze-thaw cycles.

Assay Buffer: Prepare 1×Assay Buffer by dilute 10× Assay Buffer with ddH_2O. Warm to 37°C before use.

Staining Solution: Mix 1 µl of MitoOrange™ (1000×) in each 1 ml of Assay Buffer. Scale up accordingly for larger numbers of assays.

Recommended procedures

A. Quantification by Flow Cytometry

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

1. For non-adherent cells, Collect 1-5 ×10^5 cells by centrifugation (4°C, 300 g, 5min). 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 500 uL Staining Solution.

3. Incubate the cells at 37°C for 15-45 minutes in the dark.

4. Centrifuge cells at 500 g and discard supernatant.

5. Wash cell pellet with PBS and repeat step 5.

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 Microscopy

1. For suspension cells: Follow the protocol for flow cytometry from step1 to step6 and place the cell suspension from Step A.6 on a glass slide. Cover the cells with a glass coverslip. Analyze cells by fluorescence microscopy using the appropriate filters as soon as possible.

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°C for at least 24 hours before treatment.

2.2. Wash cells with PBS twice.

2.3. Add 0.5 mL of Staining solution to cells and incubate at 37°C for 30 minutes 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 using the appropriate filters as soon as possible (Ex/Em = 579/599 nm).