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

# TraKine™ Mitochondrion Staining Kit (Green Fluorescence)

Cat #: KTC4003 Size: 100 T/500 T/2000 T

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|---|---|---|---|
| | **Mitochondrion Staining Kit (Green Fluorescence)** | | |
| REF | **Cat #:** KTC4003 | LOT | **Lot #:** Refer to product label |
| | **Fluorescence excitation/emission:** Green fluorescent probe (Ex/Em=490/523 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 (Green Fluorescence) is a set of fluorescence imaging tools to label mitochondria of live cells, the kit uses a proprietary mitochondrial green fluorescent probe (Ex/Em=490/523nm), which, unlike other probes, appears to localize to mitochondria, much less depending on mitochondrial membrane potential. The dye, a hydrophobic compound, has the added advantage that it is essentially nonfluorescent in aqueous solutions and only becomes fluorescent once it accumulates in the lipid environment of mitochondria, after fixed and permeabilized, the fluorescent signal will be lost, so only live cells can be stained. 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 | |
| MitoGreen™ (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
- Phosphate buffered saline (PBS) (pH 7.4)

## Reagent Preparation

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

**1×Assay Buffer (10×):** 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 MitoGreen™ should be determined individually.**

**A. Quantification by Flow Cytometry**

**Note: We recommend keeping unstained control cells (without MitoGreen™) 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 FL1).

**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 Invert coverslip on a glass slide and visualize cells fluorescence microscopy (Ex/Em=490/523 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) |
| KTC4004 | TraKine™ Mitochondrion Staining Kit (Orange 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.