

Cell Navigator™ Mitochondrion Staining Kit

Orange Fluorescence

Ordering Information	Storage Conditions	Instrument Platform
Product Number: 22667 (500 assays)	Keep in freezer and protect from light	Fluorescence microscope

Introduction

Mitochondria are membrane-enclosed organelles found in most eukaryotic cells. Mitochondria are sometimes described as “cellular power plants” because they generate most of the cellular supply of ATP. In addition to supplying cellular energy, mitochondria are involved in a range of other processes, such as signaling, cellular differentiation, cell death, as well as the control of the cell cycle and cell growth. Mitochondria have been implicated in several human diseases, including mitochondrial disorders and cardiac dysfunction, and may play a role in the aging process. Although most of a cellular DNA is contained in the cell nucleus, the mitochondrion has its own independent genome.

Our Cell Navigator™ fluorescence imaging kits are a set of fluorescence imaging tools for labeling sub-cellular organelles such as membranes, lysosomes, mitochondria, nuclei, etc. The selective labeling of live cell compartments provides a powerful method for studying cellular events in a spatial and temporal context.

This particular kit uses a proprietary dye that selectively accumulates in mitochondria probably via the mitochondrial membrane potential gradient. The orange fluorescent mitochondrial stain has Ex/Em = 545/575 nm (TRITC filter-compatible). The mitochondrial indicator, a hydrophobic compound, easily permeates intact live cells and trapped in mitochondria after it gets into cells. This fluorescent mitochondrial indicator is retained in mitochondria for a long time since it carries a cell-retaining group. This key feature significantly increases the staining efficiency. The kit can be readily adapted for many different types of fluorescence platforms, such as microplate assays, immunocytochemistry and flow cytometry.

Kit Components

Components	Amount
Component A: Mitolite™ Orange	100 µL (500X DMSO stock solution)
Component B: Live Cell Staining Buffer	50 mL

Assay Protocol

Brief Summary

**Prepare cells → Add dye working solution → Incubate at 37 °C for 30 minutes to 2 hours
→ Analyze under fluorescence microscope at Ex/Em = 540/590 nm (TRITC filter set)**

1. Prepare mitochondrial staining solution:

- 1.1 Warm all the components to room temperature.
- 1.2 Prepare dye working solution by diluting 20 µL of Mitolite™ Orange (Component A) into 10 mL of Live Cell Staining Buffer (Component B).

Note 1: 20 µL of 500X Mitolite™ Orange (Component A) is enough for one 96-well plate. Aliquot and store unused 500X Mitolite™ Orange at ≤ -20 °C. Protect from light and avoid repeated freeze-thaw cycles.

Note 2: The optimal concentration of the fluorescent mitochondrial indicator varies depending on the specific application. The staining conditions may be modified according to the particular cell type and the permeability of the cells or tissues to the probe.

2. Prepare and stain cells:

- 2.1 **For adherent cells:** Grow cells either in a 96-well black wall/clear bottom plate or on cover-slips inside a petri dish filled with the appropriate culture medium. When cells reach the desired confluence, add equal volume (e.g. 100 μ L for a 96-well plate and 25 μ L for a 384-well plate) of the dye-working solution (from Step 1.2). Incubate the cells in a 37 °C, 5% CO₂ incubator for 30 minutes to 2 hours. Replace the dye-loading solution with Hanks and 20 mM Hepes buffer (HH buffer) or buffer of your choice (e.g. the buffer with growth medium at 1:1 concentration). Observe the cells by using a fluorescence microscope fitted with a TRITC filter set.

Note: It is recommended to increase either the labeling concentration or the incubation time to allow the dye to accumulate if the cells do not appear to be sufficiently stained.

- 2.2 **For suspension cells:** Centrifuge the cells at 1000 rpm for 5 minutes to obtain a cell pellet and aspirate the supernatant. Resuspend the cell pellets gently in pre-warmed (37 °C) growth medium, and add equal volume of the dye-working solution (from Step 1.2). Incubate the cells in a 37 °C, 5% CO₂ incubator for 30 minutes to 2 hours. Replace the dye-loading solution with Hanks and 20 mM Hepes buffer (HH buffer) or buffer of your choice (e.g. the buffer with growth medium at 1:1 concentration). Observe the cells using a fluorescence microscope fitted with a TRITC filter set.

Note 1: It is recommended to increase either the labeling concentration or the incubation time to allow the dye to accumulate if the cells do not appear to be sufficiently stained.

Note 2: Suspension cells may be attached to cover-slips that have been treated with BD Cell-Tak® (BD Biosciences) and stained as adherent cells (see Step 2.1).

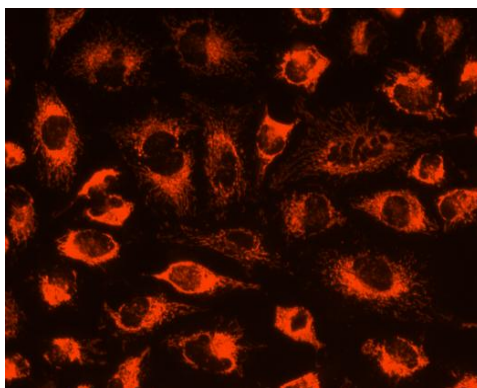


Figure 1. Image of U2OS cells stained with the Cell Navigator™ Mitochondrial Staining Kit *Orange Fluorescence* in a Costar black 96-well plate

References

1. Hung, H; Deerinck, TJ; Ellisman, MH; and Spector, DL. (1994) In vivo analysis of the stability and transport of nuclear poly(A)⁺ RNA. *J Cell Biol* 126, 877-899.
2. Barasch J, Kiss B, Prince A, Saiman L, Gruenert D, al-Awqati Q. (1991) Defective acidification of intracellular organelles in cystic fibrosis. *Nature* 1991; 352:70-73.
3. Jiang, LW; Maher, VM; McCormick, JJ and Schindler, M. (1990) Alkalinization of the lysosomes is correlated with ras transformation of murine and human fibroblasts. *J Biol Chem* 265, 4775-4777.
4. Griffiths, G; Hoflack, B; Simons, K; Mellman, I; Kornfeld, S. (1988) The mannose 6-phosphate receptor and the biogenesis of lysosomes. *Cell*. 12;52(3):329-341.

Warning: This kit is only sold to end users. Neither resale nor transfer to a third party is allowed without written permission from AAT Bioquest. Chemical analysis of the kit components is strictly prohibited. Please call us at 408-733-1055 or e-mail us at info@aatbio.com if you have any questions.