Chromeo™ Live Cell Mitochondrial Staining Kit

(version A1)

Catalog No. 15005

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Introduction

The Chromeo™ Live Cell Mitochondrial stain is based on a proprietary, non-toxic, water-soluble, membrane-permeable dye that rapidly diffuses into live or fixed cells. In live cells it stains mitochondria with low background and little or no staining of other organelles. When fixed cells are stained, internal membranes and nucleoli become additional targets of the dye.

The unique dye is not fluorescent until after it has reacted covalently with an amino group in the cell, which induces a structural change that results in bright fluorescence. This eliminates background that might be caused by any unbound dye. It has the added advantage that the dye tends to persist for a considerable period of time at the location of the reaction, specifically the mitochondria. Unlike other fluorescent stains, the Chromeo Live Cell Mitochondrial stain does not affect cell growth, is cell permeable, has a long Stokes shift, and becomes fluorescent when it accumulates in the mitochondria. There is no link between mitochondrial membrane potential and the staining pattern.

The Mitochondrial Stain can be excited between 470 nm and 550 nm (maximum at 503 nm), enabling the use of common lasers or other light sources. A principal characteristic of the dye is its long Stokes shift of about 100 nm with an emission maximum at 610 nm. The supplied Hoechst stain, with 350 nm excitation and 461 nm emission maxima, can serve as a nuclear counter-stain.

Long-term cellular labeling

In addition to serving as a mitochondrial stain, the dye can be used as a long-term cell label. Because of its excellent retention within the cell and low toxicity, the stain is an ideal tool for long-term labeling of cells and cellular tracking.

product	format	catalog no.
Chromeo™ Live Cell Mitochondrial Staining Kit	1 kit	15005

Chromeo™ Live Cell Mitochondrial Staining Kits are for research use only. Not for use in diagnostic procedures.

Overview

Mitochondria play an important role within living cells, as they are involved in a variety of cellular processes. In addition to their "main" function to generate cellular energy, mitochondria are involved in the regulation of cell growth and differentiation; they play a critical role in the induction of the apoptotic process, and are part of signaling cascades such as calcium signaling.

The structural characteristics of mitochondria are variable and depend on the organism, the cell type and the metabolic state of the individual cell. Cell cycle-dependent changes in mitochondrial morphology have also been reported. The observed structures vary between a fragmented appearance, where a high number of oval mitochondria are present within the cell, and a morphology akin to a mitochondrial web throughout the cytoplasm. Furthermore, the number of mitochondria is variable between certain cell types or the cell cycle state of the cells. Metabolically active cells such as hepatocytes are rich in mitochondria; trained muscle cells have a higher mitochondrial content than untrained muscle cells.

Mitochondria are responsible for generating over 90% of the energy needed to sustain life and support growth. Diseases of the mitochondria result in underproduction of energy within the cell, causing cell injury and even cell death. Cells of the brain, heart, liver, skeletal muscles, kidney and the endocrine and respiratory systems are the principal tissues affected by mitochondrial diseases.

Kit Performance

A typical mitochondrial staining experiment using the kit with live HeLa cells is shown below.

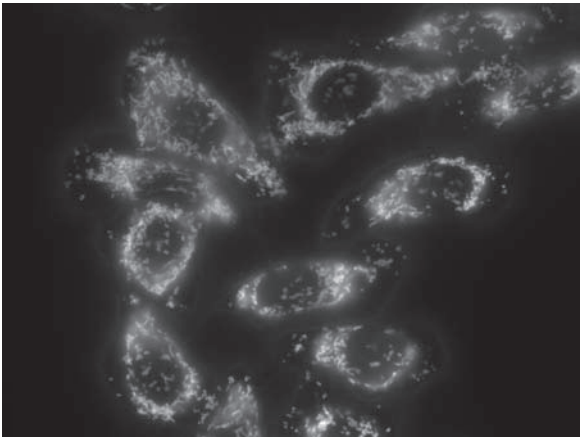

Figure 1: Mitochondria in living HeLa cells stained with the Chromeo™ Live Cell Mitochondrial Staining Kit.

Kit Components and Storage

Please store each component at the temperature indicated in the table below and protect from light, if indicated.

Reagents	Quantity	Storage / Stability
Chromeo™ Mitochondrial Cell Stain, lyophilized powder	1 vial	-20°C for 6 months in the dark
Solubilization Buffer	100 µl	-20°C for 6 months
Hoechst Nuclear Stain (1 mM)	2 x 300 µl	-20°C for 6 months in the dark

Additional materials required

- Tissue culture supplies
- Serum-free cell culture medium
- Fluorescence instrumentation

Optional materials

- 100% methanol for fixation
- Dulbecco's PBS (D-PBS), which contains Ca^{2+} and Mg^{2+}
- Glucose

PLEASE READ THE ENTIRE PROTOCOL BEFORE STARTING!

Note: Dilutions and quantities provided are guidelines. You may need to vary conditions to obtain optimal results in your specific system.

A. Preparation of the Stock Solution

To prepare the Stock Solution, add 30 µl Solubilization Buffer into the vial containing the Chromeo Live Cell Mitochondrial Stain and mix carefully with the pipette tip. This 10 mM stock solution, which will have a blue color, should be stored in aliquots at -20°C in the dark prior to use. Please avoid repeated freeze-thaw cycles.

B. Preparation of the Staining Solutions

To prepare the staining solution, dilute the appropriate volume of dye Chromeo Mitochondrial Stain Stock Solution in PBS (fixed cells) or serum-free cell culture medium (live cells). Also, if you will be performing live cell staining, the staining solution (and regular PBS for the washes) should be warmed to 37°C before beginning the procedure.

In general, dilutions from 1:10000 to 1:20000 result in the best mitochondrial staining, but optimization of the concentration may be required for different cell lines or for primary cells.

For nuclear counterstaining, Hoechst is typically added to live cells at a concentration of 1-5 µM, although the concentration may need to be optimized for a particular cell type. For a 1 µM final concentration, prepare a 1:1000 dilution (*i.e.* to each ml of medium add 1 µl of stock solution). The mitochondrial stain and Hoechst may be added to the same solution and incubated with the cells simultaneously.

As an alternative to using serum-free medium with live cells, the staining solution can be prepared in Dulbecco's PBS (D-PBS), which contains Ca^{2+} and Mg^{2+}, supplemented with 10 mM glucose. It is recommended that the glucose be added to the D-PBS immediately before each experiment and that glucose-containing D-PBS not be stored for later use. Use of D-PBS supplemented with glucose is recommended to maintain the health of live cells.

For fixed cells and live cells that will be imaged immediately after the staining procedure and then discarded, pure PBS can be used to prepare the staining solutions.

C. Cell Staining

Note: To ensure the quality of the staining and to maintain the stability of the dye, minimize light exposure of the stained cells as much as possible.

Live Cells

1. Grow cells to desired confluence on suitable coverglass or clear-bottom plates.
2. Prior to staining, wash cells twice with warm (37°C) PBS.
3. Add the warm (37°C) stain solution (in serum-free medium or D-PBS supplemented with glucose) to cells and incubate for 30 minutes in a cell-culture incubator. Ensure the entire plate surface is covered with stain, which is approximately 1 ml/well for a 6-well plate or 100 µl/well for a 96-well plate. Protect from light during the incubation.
4. Wash cells twice with warm (37°C) PBS.
5. Image the cells in PBS or cell culture medium. Depending on the imaging system to be used, medium without phenol red may be required to avoid background fluorescence.
6. If cells are not to be imaged immediately, add fresh cell culture medium and return cells into the incubator until needed.

Fixed Cells

1. Grow cells to desired confluence and wash twice with cold PBS.
2. Fix cells by adding 100% ice-cold methanol and placing the plate at -20°C for 10 minutes.
3. Wash cells twice with PBS.
4. Add the stain solution (in PBS) to cells and incubate for 30 minutes at room temperature. Ensure the entire plate surface is covered with stain, which is approximately 1 ml/well for a 6-well plate or 100 µl/well for a 96-well plate. Protect from light during the incubation.
5. Wash cells twice with PBS, then image.

D. Image Acquisition

Analyze the stained cells by fluorescence microscopy. To detect the Hoechst stained nuclei, a standard DAPI filter set (370-410nm/435-485 nm) can be used. To detect the mitochondria in combination with the Hoechst stain, a commonly used Cy3 filter set (550-580nm/590-650nm) will separate the fluorescent spectra.

In general, the broad absorption and emission peaks of the mitochondrial stain allow much flexibility for detection and the choice of commonly used filter sets.

For long-term labeling of live cells, we recommend choosing a short exposure time for each acquisition to prevent photo bleaching of the dye.

Appendix

Section A. Troubleshooting Guide

Problem/question	Possible cause	Recommendation
Staining differs across cell population	Individual cells may vary in the amount and the morphology of their mitochondria.	
Staining differs between different cell types.	The amount and the morphology of mitochondria differs between cell types.	
Staining differs between different cell types.	The amount of stain necessary to get optimal mitochondrial staining may vary between different cell types.	Optimize the staining experiment by using different dilutions of the dye stock solution.
Background staining is present.	Cell culture medium containing phenol red was used during imaging.	Wash with PBS, then use PBS to image the cells.
Intensity of the stain is dim	Cell culture medium containing serum was used during the staining reaction.	Use (supplemented) PBS or serum-free culture medium to prepare the staining solution.

Section B. Related Products

Fluorescent Cell Stains	Format	Catalog No.
LavaCell™ Live Cell Membrane Staining	200 µg	15004
Chromeo™ Red Fluorescent Fixed Cell Staining Kit	1 kit	15006

Fluorescent Dyes	Excitation / Emission	Format	Catalog No.
Chromeo™ 488 Carboxylic Acid	488 nm / 517 nm	1 mg	15510
Chromeo™ 488 NHS-Ester	488 nm / 517 nm	1 mg	15511
Chromeo™ 494 Carboxylic Acid	494 nm / 628 nm	1 mg	15110
Chromeo™ 494 NHS-Ester	494 nm / 628 nm	1 mg	15111
Chromeo™ 505 Carboxylic Acid	505 nm / 526 nm	1 mg	15610
Chromeo™ 505 NHS-Ester	505 nm / 526 nm	1 mg	15611
Chromeo™ 546 Carboxylic Acid	545 nm / 561 nm	1 mg	15210
Chromeo™ 546 NHS-Ester	545 nm / 561 nm	1 mg	15211
Chromeo™ 642 Carboxylic Acid	642 nm / 660 nm	1 mg	15310
Chromeo™ 642 NHS-Ester	642 nm / 660 nm	1 mg	15311

Antibody/Protein Labeling	Excitation / Emission	Format	Catalog No.
Chromeo™ 488 Antibody Labeling Kit	488 nm / 517 nm	1 kit	15090
Chromeo™ 494 Antibody Labeling Kit	494 nm / 628 nm	1 kit	15091
Chromeo™ 546 Antibody Labeling Kit	545 nm / 561 nm	1 kit	15092
Chromeo™ 642 Antibody Labeling Kit	642 nm / 660 nm	1 kit	15093

MAX Stain™ Immunofluorescence Tools	Format	Catalog No.
MAXpack™ Immunostaining Media Kit (contains 1 each of 15252, 15253 & 15254)	1 kit	15251
MAXblock™ Blocking Medium	150 ml	15252
MAXbind™ Staining Medium	250 ml	15253
MAXwash™ Washing Medium	1000 ml	15254

Fluorescent Secondary Antibodies	Format	Catalog No.
Chromeo™ 488 Goat anti-Mouse IgG	1 mg	15031
Chromeo™ 488 Goat anti-Rabbit IgG	1 mg	15041
Chromeo™ 494 Goat anti-Rabbit IgG	1 mg	15042
Chromeo™ 546 Goat anti-Mouse IgG	1 mg	15033
Chromeo™ 546 Goat anti-Rabbit IgG	1 mg	15043
Chromeo™ 642 Goat anti-Mouse IgG	1 mg	15034
Chromeo™ 642 Goat anti-Rabbit IgG	1 mg	15044
ATTO 594 Goat anti-Mouse IgG	250 µl	15037
ATTO 594 Goat anti-Rabbit IgG	250 µl	15047
ATTO 647N (STED) Goat anti-Mouse IgG	250 µl	15038
ATTO 647N (STED) Goat anti-Rabbit IgG	250 µl	15048
ATTO 655N (STED) Goat anti-Mouse IgG	250 µl	15039
ATTO 655N (STED) Goat anti-Rabbit IgG	250 µl	15049

Fluorescent Cell Viability Assay	Format	Catalog No.
ToxCount™ Cell Viability Assay	20 x 96 rxns	18010

Fluorescent Protein Labeling	Format	Catalog No.
LigandLink™ pLL-1 Kit	1 kit	34001
LigandLink™ pLL-1-NFκB p65 Kit	1 kit	34004
LigandLink™ pLL-1-p53 Kit	1 kit	34005
LigandLink™ pLL-1-STAT1 Kit	1 kit	34006
LigandLink™ Fluorescein Label	300 rxns	34101
LigandLink™ Hexachlorofluorescein Label	300 rxns	34104

Luciferase Assays	Format	Catalog No.
RapidReporter™ Gaussia Luciferase Assay	100 rxns	33001
	1000 rxns	33002
RapidReporter™ pRR-High vector	10 µg	33003
RapidReporter™ pRR-High Assay	100 rxns	33004
RapidReporter™ pRR-Low vector	10 µg	33005
RapidReporter™ pRR-Low Assay	100 rxns	33006
RapidReporter™ pRR-High-CRE vector	10 µg	33007
RapidReporter™ pRR-High-CRE Assay	100 rxns	33008
RapidReporter™ pRR-High-GR vector	10 µg	33011
RapidReporter™ pRR-High-GR Assay	100 rxns	33012
RapidReporter™ pRR-High-IRF-1 vector	10 µg	33017
RapidReporter™ pRR-High-IRF-1 Assay	100 rxns	33018
RapidReporter™ pRR-High-NFκB vector	10 µg	33009
RapidReporter™ pRR-High-NFκB Assay	100 rxns	33010
RapidReporter™ pRR-High-STAT1 vector	10 µg	33015
RapidReporter™ pRR-High-STAT1 Assay	100 rxns	33016
RapidReporter™ pRR-High-STAT3 vector	10 µg	33013
RapidReporter™ pRR-High-STAT3 Assay	100 rxns	33014

Technical Services

If you need assistance at any time, please call Active Motif Technical Service at one of the numbers listed below.

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