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ab219939 CytoPainter Mitochondrial Staining Kit - Blue Fluorescence

For staining mitochondria in live cells using our proprietary MitoBlue Indicator fluorescence probe

This product is for research use only and is not intended for diagnostic use.

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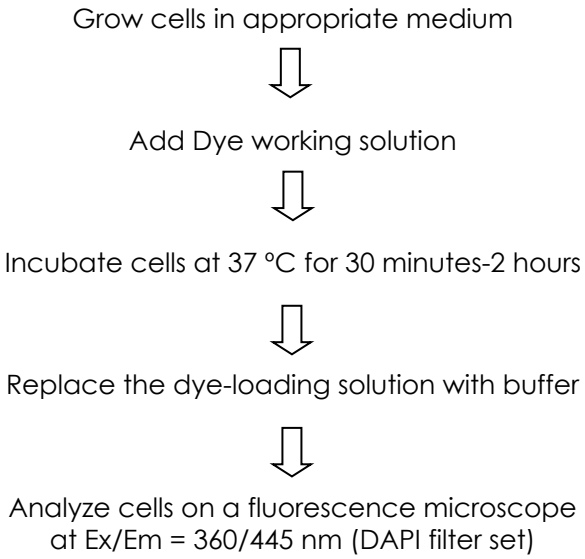
1. Overview

CytoPainter Mitochondrial Staining Kit - Blue Fluorescence (ab219939) uses a proprietary fluorescent blue dye that selectively accumulates in mitochondria in live cells and can be detected at Ex/Em = 360/445 nm (DAPI filter-compatible). The mitochondrial indicator is a hydrophobic compound that easily permeates intact live cells and becomes trapped in mitochondria, where it can be retained in mitochondria for a long time thanks to its cell-retaining group. The kit can be easily adapted for many different types of fluorescence platforms, such as microplate assays, and flow cytometry.

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.

2. Protocol Summary



3. Precautions

Please read these instructions carefully prior to beginning the assay.

- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Assay kit 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.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
MitoBlue Indicator (500X DMSO stock solution)	100 µL	-20°C	-20°C
Live Cell Staining Buffer	50 mL	-20°C	-20°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Fluorescence microscope fitted with a filter capable of detecting fluorescence at Ex/Em = 360/445 nm (DAPI filter set)
- HHBS (Hank's Balanced Salt solution + 20 mM HEPES buffer)
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- General tissue culture supplies
- Sterile 96 well plate with clear flat bottom, preferably black (if performing assay in microplate format). Use a poly-D-lysine coated plate for suspension cells

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample and reagent additions.
- 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.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 Live Cell Staining Buffer (50 mL):

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.2 MitoBlue Indicator (500X):

Ready to use as supplied. Equilibrate to room temperature before use. Aliquot MitoBlue Indicator so that you have enough volume to perform the desired number of assays. Store at -20°C protected from light. Avoid repeated freeze-thaw cycles.

10. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all controls and samples in duplicate.
- Each cell line should be evaluated on an individual basis to determine the optimal cell density.

Δ Note: The optimal concentration and incubation time of the MitoBlue Indicator will vary depending on the specific application. The staining conditions may be modified according to the particular cell type and/or the permeability of the cells or tissues to the probe.

10.1 Prepare Mitochondrial Staining Solution:

10.1.1 Prepare dye working solution by diluting 20 µL of 500X MitoBlue Indicator Stock solution (Step 9.2) into 10 mL of Live Cell Staining Buffer.

Δ Note: 20 µL of 500X Blue Dye is enough for one 96 well plate.

10.1.2 Proceed to step 10.2 for adherent cell staining protocol or step 10.3 for suspension cell staining protocol.

10.2 Adherent cell staining:

10.2.1.1 Grow cells in a 96 well black wall/clear bottom plate with the appropriate culture medium.

Δ Note: cells can also be grown cover-slips inside a petri dish. In that case, you will need to modify the volume of the staining solution accordingly to the final volume.

10.2.1.2 When cells reach the desired confluence (70-80%), add equal volume (100 µL/well) of the Mitochondrial dye-working solution.

10.2.1.3 Incubate the cells for 30 minutes-2 hours in a 37°C/5% CO_2 incubator.

10.2.1.4 Aspirate dye-loading solution carefully.

10.2.1.5 Add 100 µL of HHBS buffer or buffer of your choice (e.g. the buffer with growth medium at 1:1 concentration).

10.2.1.6 Observe the cells by using a fluorescence microscope fitted with a DAPI filter set (Ex/Em = 360/445 nm).

10.3 Suspension cell staining:

10.3.1.1 Grow cells in the appropriate culture vessel until the reach the desired confluence (70-80%).

Δ Note: Suspension cells may be attached to microplate or coverslips that have been treated with poly-D-lysine can be stained following the procedure for adherent cells (Step 10.2).

10.3.1.2 Centrifuge suspension cells at 1,000 rpm for 5 minutes to obtain a cell pellet and aspirate the supernatant.

10.3.1.3 Resuspend the cell pellets gently in pre-warmed (37°C) growth medium.

10.3.1.4 Add equal volume of the Mitochondrial dye-working solution (Step 10.1) to resuspended cells and transfer to microplate or coverslips.

10.3.1.5 Incubate the cells for 30 minutes-2 hours in a 37°C/5% CO_2 incubator.

10.3.1.6 Replace the Mitochondrial dye-loading solution with HHBS buffer or buffer of your choice (e.g. the buffer with growth medium at 1:1 concentration).

10.3.1.7 Observe the cells by using a fluorescence microscope fitted with a DAPI filter set (Ex/Em = 360/445 nm).

11. Data Analysis

- We recommend acquiring several images per well.
- We recommend data analysis after coding and mixing images to ensure unbiased results.
- For manual analysis, if you do not have a specific software installed in your microscope, you can download ImageJ, an open source image processing designed for scientific multidimensional images by the National Institute of Health (NIH).

12. Typical Data

Data provided for **demonstration purposes** only.

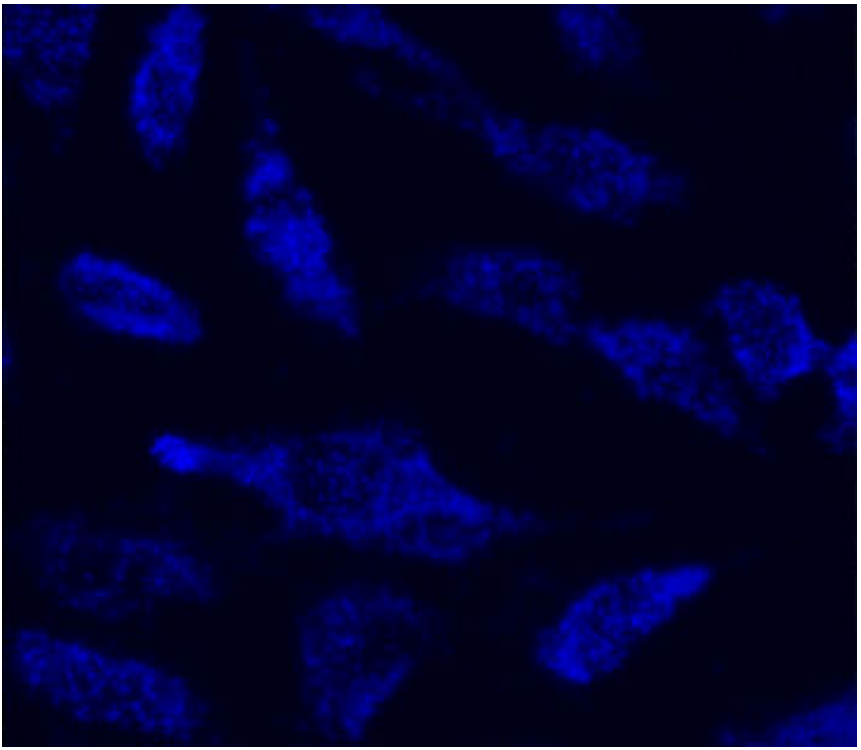


Figure 1. HeLa cells stained with CytoPainter Mitochondrial Staining Kit - Blue Fluorescence (ab219939) in a 96 well clear-bottom plate.

13. Notes

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