MitoDNA™ Blue 470

Catalog number: 22684
Unit size: 1 mg

Component	Storage	Amount (Cat No. 22684)
MitoDNA™ Blue 470	Freeze (< -15 °C), Minimize light exposure	1 mg

OVERVIEW

There are very few probes that can be effectively used to detect mitochondrial deoxyribonucleic acid (mtDNA). The common fluorescent DNA probes (such as DAPI, Hoechst or SYBR® Green) lack the specificity to target mitochondria. They predominantly stain nuclei. MitoDNA™ Blue 470 is a cell permeable dye that specifically stains mtDNA in live cells. It provides an efficient way of labeling mtDNA for the dynamic imaging of mtDNA in live cells. MitoDNA™ Blue 470 has a good Stokes Shift that gives great signal-to-noise ratio and can be easily used for multiplex staining in cells with other fluorescent imaging probes. mtDNA is a small circular DNA found within mitochondria present in the cytoplasm of a cell. This DNA is supplementary to the nucleic acid material found in the nucleus of each cell. The mtDNA codes for 37 genes that promote the proper functioning of some cells. The mitochondria synthesize adenosine triphosphate (ATP) through oxidative phosphorylation and encode information for the synthesis of enzymes, transfer ribonucleic acid (tRNA), and ribosomal RNA (rRNA). Disorders of mtDNA and mutations in its genes can predispose to health problems like age-related hearing loss, diabetes, and brain, heart, and liver failure, among other conditions. Moreover, mtDNA and its associated mitochondrial disorders can predispose people to different types of cancers including lymphomas, leukemias, and breast, intestine, liver and kidney tumors etc.

AT A GLANCE

Important Note

Before using MitoDNA™ Blue 470 for the first time, allow it to thaw at room temperature. Then, briefly centrifuge it to collect the dried pellet.

Protocol Summary

1. Prepare cells in a growth medium.
2. Stain cells with MitoDNA™ Blue 470 working solution.
3. Incubate samples for 5 to 15 minutes at 37 °C.
4. Use a fluorescence microscope with a DAPI filter set to monitor fluorescence intensity.

KEY PARAMETERS

Fluorescence microscope

Emission	DAPI Filter
Excitation	DAPI Filter
Recommended plate	Black wall/clear bottom

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles

MitoDNA™ Blue 470 Stock Solution

1. Prepare a 5 to 10 mM MitoDNA™ Blue 470 stock solution in DMSO. For example, add 150 µL of DMSO to the MitoDNA™ Blue 470 vial to create a 10 mM stock solution.

   Note: Prepare a single aliquot of the unused MitoDNA™ Blue 470 stock solution and store it at ≤ -20 º C, protected from light. Avoid repeated freeze-thaw cycles.

PREPARATION OF WORKING SOLUTION

MitoDNA™ Blue 470 Working Solution

1. Prepare a 5 to 10 µM working solution by diluting the MitoDNA™ Blue 470 stock solution in Hanks' solution with 20 mM HEPES buffer (HHBS).

   Note: For optimal results, use this solution within a few hours of preparation.

   Note: Cover the working solution with foil or store it in a dark place to protect it from light.

SAMPLE EXPERIMENTAL PROTOCOL

1. Plate the cells in a 96-well plate with black walls and a clear bottom.
2. Remove the cell culture medium and add 100 µL of MitoDNA™ Blue 470 working solution directly to the cells.
3. Incubate the cells at 37°C for 5-15 minutes, protected from light.

   Note: The concentration and incubation time of MitoDNA™ Blue 470 may vary depending on the cell line. Test different concentrations to determine the optimal dose.

4. Remove the dye working solution and wash the cells twice with HHBS buffer.
5. Add HHBS buffer and analyze the cells using a fluorescence microscope equipped with a DAPI filter set.

EXAMPLE DATA ANALYSIS AND FIGURES

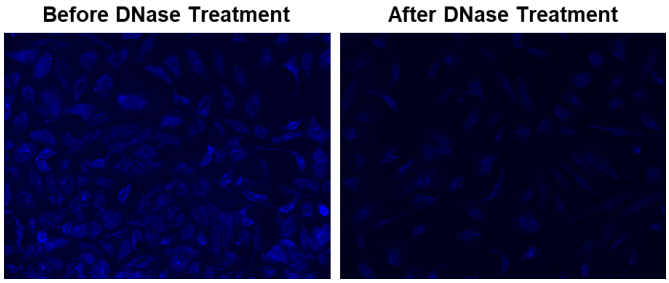

Figure 1. Fluorescence response of MitoDNA™ Blue 470 (5 µM) before and after DNase (2 units/reaction) at 37 °C for 1 hour treatment in HeLa cells. The fluorescence intensities were monitored with fluorescence microscopy.

DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email info@aatbio.com if you have any questions.