MitoDNA™ Red 680

Catalog number: 22688
Unit size: 1 mg

Component	Storage	Amount (Cat No. 22688)
MitoDNA™ Red 680	Freeze (< -15 °C), Minimize light exposure	1 mg

OVERVIEW

There are limited probes available that effectively detect mitochondrial deoxyribonucleic acid (mtDNA). Common fluorescent DNA probes, such as DAPI, Hoechst, or SYBR® Green, lack the specificity required for mitochondrial targeting, primarily staining nuclear DNA. MitoDNA™ Red 680 is a cell-permeable dye that specifically stains mtDNA in live cells, offering an efficient method for the dynamic imaging of mtDNA. This dye exhibits a large Stokes shift, providing an excellent signal-to-noise ratio and enabling easy multiplex staining with other fluorescent probes. mtDNA is a small, circular DNA molecule located within mitochondria in the cytoplasm. It is supplementary to nuclear DNA and encodes 37 genes essential for mitochondrial and cellular functions. Mitochondria are responsible for ATP synthesis through oxidative phosphorylation and contain the genetic information for synthesizing key enzymes, transfer RNA (tRNA), and ribosomal RNA (rRNA). Mutations and disorders in mtDNA can lead to a range of health issues, including age-related hearing loss, diabetes, and failures in the brain, heart, and liver. Additionally, mtDNA mutations are associated with an increased risk of various cancers, including lymphomas, leukemias, and tumors in the breast, intestines, liver, and kidneys.

AT A GLANCE

Important Note

Before using MitoDNA™ Red 680 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™ Red 680 working solution.
3. Incubate samples for 5 to 15 minutes at 37 °C.
4. Monitor fluorescence intensity at Ex/Em = 600/680 nm.

KEY PARAMETERS

Fluorescence microscope

Emission	680 nm
Excitation	600 nm
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™ Red 680 Stock Solution

1. Prepare a 5 to 10 mM MitoDNA™ Red 680 stock solution in DMSO. For example, add 205 µL of DMSO to the MitoDNA™ Red 680 vial to create a 10 mM stock solution.

   Note: Prepare a single aliquot of the unused MitoDNA™ Red 680 stock solution and store it at ≤ -20 ° C, protected from light. Avoid repeated freeze-thaw cycles.

PREPARATION OF WORKING SOLUTION

MitoDNA™ Red 680 Working Solution

1. Prepare a 5 to 10 µM working solution by diluting the MitoDNA™ Red 680 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™ Red 680 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™ Red 680 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 with excitation/emission settings of 600/680 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

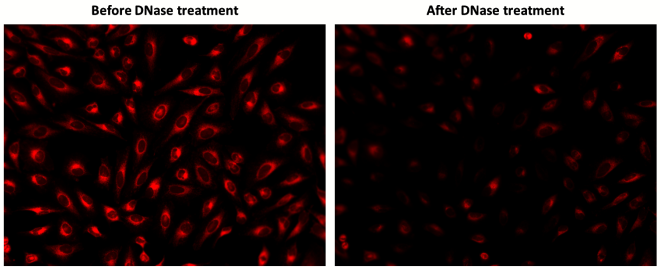

Figure 1. The fluorescence response of MitoDNA™ Red 680 (5 µM) in HeLa cells was assessed before and after DNase treatment. Fluorescence intensities were monitored using fluorescence microscopy.

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