

**MitoDNA™ Green 530**

 Catalog number: 22685  
 Unit size: 1 mg

Component	Storage	Amount (Cat No. 22685)
MitoDNA™ Green 530	Freeze (< -15 °C), Minimize light exposure	1 mg

**OVERVIEW**

Detecting mitochondrial DNA (mtDNA) has been challenging due to the non-specific nature of conventional DNA probes like DAPI, Hoechst, or SYBR® Green, which primarily stain the nucleus. MitoDNA™ Green 530 overcomes this limitation with its high specificity for mtDNA, allowing researchers to selectively visualize and track mitochondrial dynamics in live cells. This cell-permeable dye offers a large Stokes Shift, providing a high signal-to-noise ratio ideal for multiplex imaging alongside other fluorescent probes.

mtDNA is a small, circular DNA found in the mitochondria of a cell, separate from the nuclear DNA. It encodes 37 genes essential for mitochondrial function, including those involved in ATP synthesis, enzyme production, and tRNA and rRNA synthesis. Mutations or disorders in mtDNA can lead to various health issues, including age-related hearing loss, diabetes, and failures of the brain, heart, and liver. Additionally, mtDNA mutations are linked to an increased risk of cancers, such as lymphomas, leukemias, and tumors in the breast, intestine, liver, and kidneys.

**AT A GLANCE**
**Important Note**

Before initial use, thaw MitoDNA™ Green 530 at room temperature and briefly centrifuge to collect the dried pellet.

**Protocol Summary**

1. Prepare cells in growth medium.
2. Stain cells with MitoDNA™ Green 530 working solution.
3. Incubate samples at 37°C for 5–15 minutes.
4. Monitor fluorescence intensity with FITC filter.

**KEY PARAMETERS**
**Fluorescence microscope**

Emission	FITC Filter
Excitation	FITC 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™ Green 530 Stock Solution**

1. Prepare a 5–10 mM MitoDNA™ Green 530 stock solution in DMSO. For example, add 236 µL of DMSO to the MitoDNA™ Green 530 vial to create a 10 mM stock solution.

**Note:** Prepare single-use aliquots of the stock solution and store

at ≤ -20°C. Protect from light and avoid repeated freeze-thaw cycles.

**PREPARATION OF WORKING SOLUTION**
**MitoDNA™ Green 530 Working Solution**

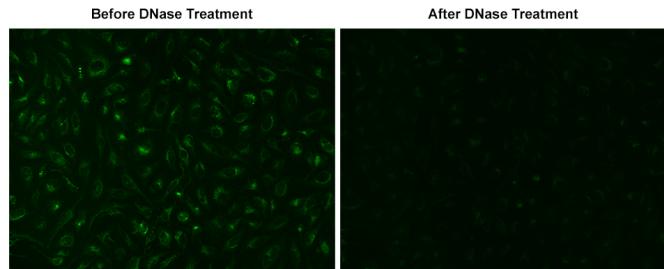
1. Prepare a 1–5 µM working solution by diluting the MitoDNA™ Green 530 stock solution in Hanks solution with 20 mM Hepes buffer (HHBS, AAT Cat# 20011).

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

**Note:** Protect the working solution from light by covering it with foil or placing it in the dark.

**SAMPLE EXPERIMENTAL PROTOCOL**

1. Plate cells as needed in a 96-well black wall, clear bottom plate.
2. Remove the cell culture medium and add 100 µL of MitoDNA™ Green 530 working solution to each well.
3. Incubate cells at 37°C in a 5% CO<sub>2</sub> incubator for 5–15 minutes, keeping them protected from light.
4. Remove the dye working solution and wash the cells twice with HHBS buffer.
5. Add HHBS buffer and analyze cells using a fluorescence microscope with a FITC filter set.

**EXAMPLE DATA ANALYSIS AND FIGURES**


**Figure 1.** Fluorescence response of MitoDNA™ Green 530 (0.2 µM) in HeLa cells before and after treatment with DNase (2 units/reaction) at 37°C for 1 hour. Fluorescence intensities were monitored using fluorescence microscopy.

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