MITO-ID® Membrane potential cytotoxicity kit

A Real-time Mitochondrial membrane Potential Assay with Superior Sensitivity

The MITO-ID® Membrane Potential Cytotoxicity Kit measures fluctuations in mitochondrial membrane potential (MMP) utilizing a cationic dual-emission dye that exists as green fluorescent monomers in the cytosol, and accumulates as orange fluorescent J-aggregates in the mitochondria. Mitochondria having a low membrane potential will accumulate low concentrations of dye and will exhibit green fluorescence while more highly polarized mitochondria will exhibit orange-red fluorescence. Cells exhibit a shift from orange to green fluorescence as mitochondrial function becomes increasingly compromised. The kit is a unique HTS assay that monitors mitochondrial membrane potential in real-time without the need for washes or medium removal.

Mechanism of Action

The basic chemical structure of the dye consists of highly conjugated moieties that extensively delocalize a positive charge thus allowing electrophoretic uptake toward the negatively charged matrix phase of the polarized inner mitochondrial membrane. The dye is capable of entering selectively into mitochondria wherein it changes its color reversibly from green to orange as membrane potential increases (dual-emission potential probe). This photophysical property is due to the reversible formation of J-aggregates upon membrane polarization that causes shifts in emitted light from ~530nm (the emission of the monomeric dye) to 590 nm (the emission of the J-aggregate form) when excited at 490 nm. As a consequence, mitochondria having a low membrane potential will accumulate low concentrations of dye and will exhibit green fluorescence while more highly polarized mitochondria will exhibit orange fluorescence.

Mitochondria play a central role in cellular metabolism, bioenergetics, and apoptosis. Decreased mitochondrial function is known to be a major contributor to drug-associated toxicity in various organs. Growing FDA emphasis on evaluating the mitotoxic effects of drug candidates has increased the importance of determining such effects early in the drug development process.

Citations: 32 View Online »

- 10X more sensitive than JC-1 with superior aqueous solubility
- Photostable dual-emission dye
- Suitable for time-course studies evaluating intact and compromised mitochondria
- No-wash/No-medium removal
- Separate MITO-ID® assay is available for detection of mitochondrial mass
- Detects toxicity at lower drug/dose concentrations
- No solvent artifacts as those seen with JC-1 formulation
- Suitable for high-throughput applications

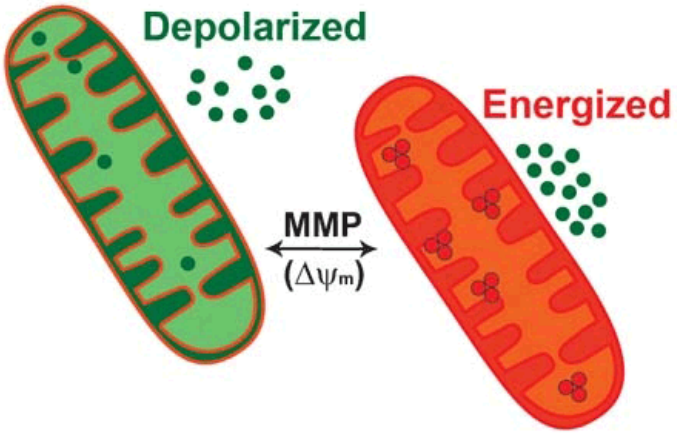
Ordering Information

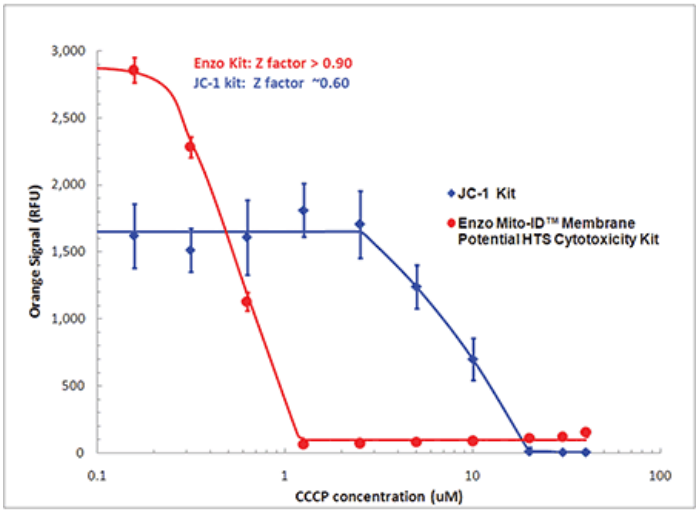
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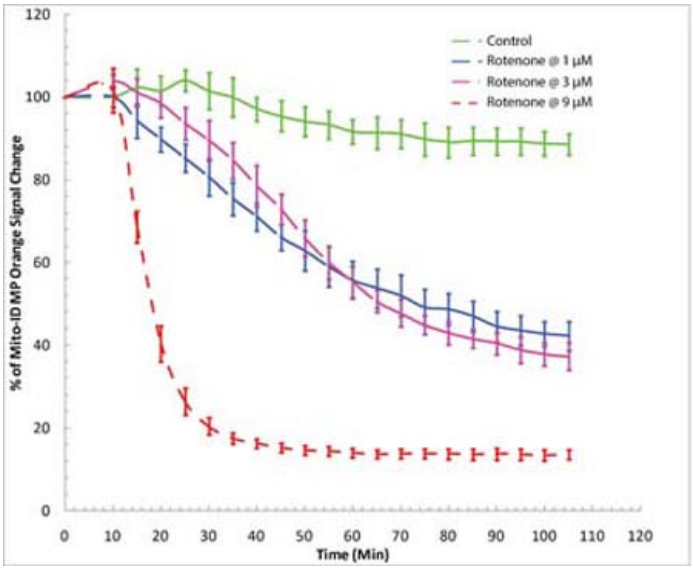
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Detect mitochondrial perturbations with 10 times more sensitivity than JC-1. Mitochondrial membrane potential (MMP) was evaluated in HeLa cells treated with CCCP using MITO-ID® dye (red) or JC-1 (blue). Using a conventional fluorescence microplate reader, MMP was shown to decrease with increasing CCCP concentration as indicated by a decrease in orange fluorescence. Improved aqueous solubility of the dye and no-wash protocol minimizes variability, leading to a higher Z-factor (> 0.9) than that obtained with JC-1.



Real-time detection of mitotoxicity in drug screening. Time-course study of mitochondrial membrane potential changes using a BioTek Synergy™ Mx fluorescence microplate reader. HeLa cells were incubated with MITO-ID® MP dye for 30 minutes at room temperature (no serum or media removal). Rotenone was added to achieve concentrations of 1 µM, 3 µM and 9 µM. MITO-ID® MP dye was shown to be responsive to rotenone, as demonstrated by a decrease in orange signal.

Handling & Storage

Handling	Protect from light. Avoid freeze/thaw cycles.
Short Term Storage	-20°C
Long Term Storage	-80°C
Shipping	Dry Ice

Regulatory Status

RUO - Research Use Only

Product Details

Application	HTS, Microplate
Application Notes	MITO-ID® Membrane Potential Cytotoxicity Kit enables monitoring of mitochondrial potential changes using a simple fluorescence microplate reader.
Contents	MITO-ID® MP Detection Reagent, 200 µL CCCP Control, 100 µL 10X Assay Buffer 1: 2.5 mL 50X Assay Buffer 2: 0.5 mL
Quality Control	A sample kit from each lot of MITO-ID® Membrane Potential Cytotoxicity Kit is assayed using the microplate-based assay described in the manual. The Z'-factor from CCCP-treated cells is greater than 0.6.
Quantity	For 2 x 96-well microplates
Technical Info / Product Notes	The MITO-ID® Membrane Potential Cytotoxicity Kit is a member of the CELLESTIAL® product line, reagents and assay kits comprising fluorescent molecular probes that have been extensively benchmarked for live cell analysis applications. CELLESTIAL® reagents and kits are optimal for use in demanding imaging applications, such as confocal microscopy, flow cytometry and HCS, where consistency and reproducibility are required.

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