Technical Brief

Catalogue No. MCH100110

Muse™ MitoPotential Assay

Early and Sensitive Detection of Cellular Health Perturbation

Assay Features

- Specific detection of mitochondrial depolarization and cell death in individual cells
- Quick determination of live, depolarized, depolarized/dead and dead cells
- No-wash, mix-and-read, rapid assay
- Simplified acquisition and analysis
- Minimal number of cells required
- Validated with both adherent and suspension cells
- Accurate and precise

Rapid, Sensitive Detection of Mitochondrial Depolarization

Mitochondria are important cellular organelles that maintain crucial cellular energy balance, are a primary site of production of free radicals and contain key regulators of cell death processes, such as apoptosis. Mitochondrial changes are thus highly sensitive indicators of cell health and stress. Cellular energy produced during mitochondrial respiration is stored as an electrochemical gradient across the mitochondrial membrane. In healthy cells, this accumulation of energy creates a mitochondrial transmembrane potential ($\Delta\Psi m$) that enables the cell to drive the synthesis of adenosine triphosphate (ATP). A decrease in the mitochondrial inner transmembrane potential is often, but not always, associated with the early stages of apoptosis. Collapse of this potential is believed to coincide with the opening of the mitochondrial permeability transition pores, leading to the release of cytochrome C into the cytosol, which then triggers the downstream events in the apoptotic cascade. Mitochondrial membrane potential changes have been implicated in apoptosis, necrotic cell death and caspase-independent cell death. Depolarization of the inner mitochondrial membrane potential is thus a reliable indicator of mitochondrial dysfunction and cellular health, which have become increasingly important in the study of apoptosis, drug toxicity and multiple disease states.

Assay Principle

The Muse™ MitoPotential Assay utilizes the MitoPotential Reagent, a cationic, lipophilic dye, to detect changes the mitochondrial membrane potential and 7-AAD as an indicator of cell death. Included in the kit are:

1) MitoPotential Reagent: High membrane potential drives accumulation of MitoPotential Reagent within the inner membrane of intact mitochondria resulting in high fluorescence. Cells with depolarized mitochondria demonstrate a decrease in fluorescence. This parameter is displayed on the MitoPotential axis.

(2) A dead cell marker (7-AAD) is also used as an indicator of cell membrane structural integrity and cell death. It is excluded from live, healthy cells, as well as early apoptotic cells. Dead cells thus show increased fluorescence in the Viability axis.

Four populations of cells can thus be distinguished in the assay

1. Live cells with intact mitochondrial membrane: MitoPotential(+) and 7-AAD(-)
2. Live cells with depolarized mitochondrial membrane: MitoPotential(-) and 7-AAD(-)
3. Dead cells with depolarized mitochondrial membrane: MitoPotential(-) and 7-AAD(+)
4. Dead cells with intact mitochondrial membrane: MitoPotential(+) and 7-AAD(+)

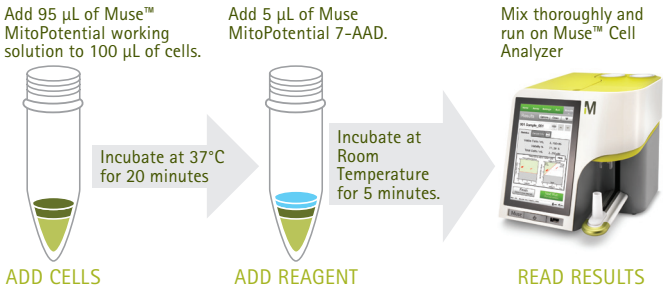

Figure 2.

The Muse™ MitoPotential Assay uses a simple mix-and-read protocol, enabling easy determination of cells exhibiting mitochondrial depolarization and cell death.

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Touchscreen Interface Greatly Simplifies Acquisition and Analysis of Apoptosis Data.

The Muse™ MitoPotential software module guides you through setup, acquisition and analysis in a few simple steps.

- Intuitive touchscreen which guides users to the answers.
- Results include count and percentage of populations automatically displayed after acquisition.
- Easy export of raw data to Excel® format enable archiving of results and additional analysis.

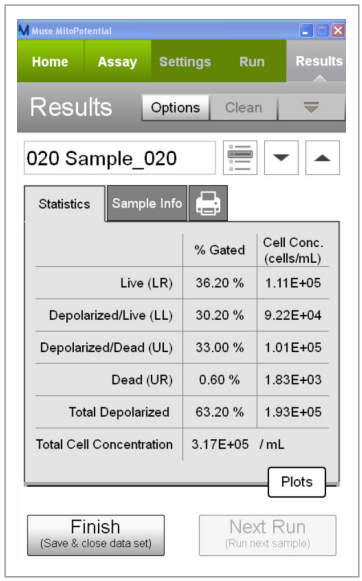

Figure 3. Results obtained using Jurkat cells treated with 2µM of staurosporine to induce mitochondrial membrane depolarization, stained with the Muse™ MitoPotential Assay and acquired on the Muse™ Cell Analyzer.

Early and Sensitive Detection of Cell Health Perturbation

The Muse™ MitoPotential enables detection of mitochondrial depolarization with multiple treatment conditions and multiple cell types as shown for cell lines treated with multiple inducers (Figure 4). Figure 5 demonstrates that the Muse™ MitoPotential Assay provides accurate quantitation of cells with depolarized mitochondria, as the data are consistent with other cytometric assays for mitochondrial depolarization.

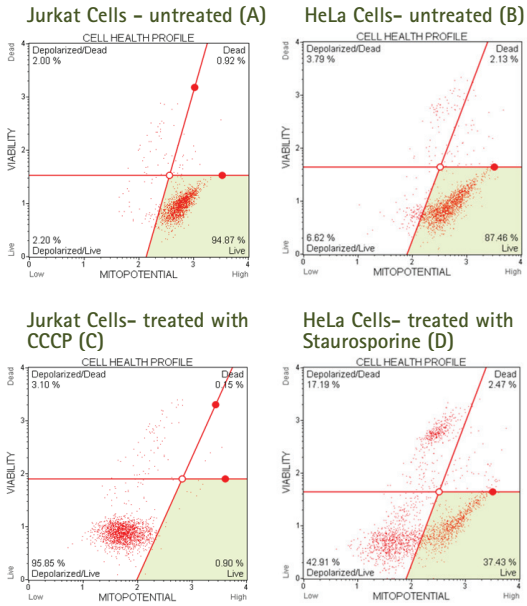

Figure 4. Impact of apoptosis-inducing compounds on Jurkat cells (suspension line) and HeLa cells (adherent line) using the Muse™ MitoPotential Assay. Dot plots show untreated cells (A and B) and cells treated with CCCP (C) and Staurosporine (D)

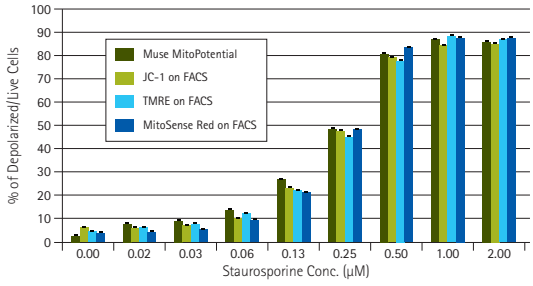

Figure 5. The Muse™ MitoPotential assay provides dose-dependent depolarization data consistent with data from other flow cytometry assays. Jurkat cells were treated with multiple concentrations of staurosporine and then stained with membrane-permeant dyes JC-1, tetramethylrhodamine ethyl ester (TMRE), or MitoPotential reagent (same as MitoSense Red). Data were acquired on either the Muse™ Cell Analyzer or FACS instrument. .

Ordering Information

Muse™ MitoPotential Assay	MCH100110	Muse™ Cell Cycle Kit	MCH100106
Muse™ Caspase-3/7 Assay	MCH100108	Muse™ Count & Viability Kit	MCH100102
Muse™ MultiCaspase Assay	MCH100109	Muse™ Annexin V & Dead Cell Kit	MCH100105
Muse™ System Check Kit	MCH100101		

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