Monitoring protein translocation, such as the release of cytochrome c and other apoptotic factors from mitochondria, reveals the nature and site of apoptotic signaling and hence the mode of action of a compound on the apoptotic machinery.

The basis of the MitoTox™ approach to monitoring apoptosis utilizes cell fractionation kits which provide rapid and simple separation of mitochondrial, cytosolic and nuclear fractions. These kits yield separation of the three compartments allowing Western blot assessment of protein movements as a result of any drug action.

Two versions of the cell fractionation kit are available, a standard version (ab109718) for use with large batches of cells and a high throughput version (ab109719) for use with cells grown in 96-well plates. A Western blotting antibody cocktail is available for observing movements of cytochrome c (ab110415) and numerous individual antibodies against other important apoptosis targets are also available. Lysates from this method can also be used in Cytochrome c Protein quantity microplate assay kit (ab110172).

A wide range of approaches are available for assessing the contribution of oxidative stress to mitochondrial toxicity. Increased ROS is an obvious indicator and other factors that could demonstrate high levels of oxidative stress include induction of apoptosis and inhibition of the respiratory chain.

Abcam has developed simple yet effective tools to take advantage of both direct and indirect approaches:

Direct: Actual modifications to proteins resulting from increased oxidation/nitration can occur. Nitration of tyrosine residues is one such modification and formation of the resulting 3-nitrotyrosine molecule are used as a direct indicator and other factors that could demonstrate high levels of oxidative stress include induction of apoptosis and inhibition of the respiratory chain.

Indirect: When free radical production is localized to the electron transport chain or other critical enzymes, enzyme activity levels are measured along with protein nitration. An indirect approach to measuring the impacts of oxidative stress is to measure the levels and activities of key antioxidant enzymes. Aconitate, catalase and SOD2 are all important contributors to ROS-reduction and therefore vitally important to measure.
Why is Testing for Mitochondrial Toxicity Important?

- Mitochondria perform two critical functions in the cell, namely the production of more than 90% of the cell's energy and the control of cell survival as an integral part of programmed cell death (apoptosis).
- Evaluating compounds for mitochondrial toxicity is an important capability for most drug safety programs and there is now much more focus on identifying mitochondrial toxicity early in the development process.

Common Drug-Induced Effects on Mitochondria

- Reduced membrane potential
- Most effects can be both direct and indirect responses to drug activity.
- Altered oxygen consumption
- Increased extracellular acidification
- Reduced mitochondrial DNA-encoded protein expression
- Increased intracellular reactive oxygen species (ROS)
- Reduced intracellular ATP levels

Mitochondrial Function

Discover more at abcam.com/MitoSciences

Screening

Basic mitochondrial function can be assessed using the Luminometric ATP Detection JC-1 Membrane Potential and DCDFA Cellular Reactive Oxygen Species Kits and can be used on a variety of platforms, including flow cytometry and fluorescent plate reader. Protocols are available for preparing cells for maximum sensitivity to mitochondrial toxins, and large published datasets allow for comparative analysis.

For antiviral and antibiotic drugs, a MitoBiogenesis™ assay is the ideal tool to uncover chronic effects on mitochondrial DNA replication and protein synthesis - both in-Cell ELISA and Western blotting antibody cocktails are available.

An apoptosis assay for screening, such as cleaved PARP, is a useful indicator of mitochondria-mediated apoptosis and can be measured sensitively in In-Cell ELISA.

Oxygen consumption and lactate production provide additional confirmation of mitochondrial liabilities and potential compensating effects.

MitoTox™ family of assays provide a complete range of solutions for all stages of mitochondrial safety analysis and measurement of the key parameters of mitochondrial function.

Screening for mitochondrial toxicity allows investigators to obtain data to establish the multiple inhibitory effects induced by mitochondrial toxins.

If mitochondrial toxicity is confirmed during in-vitro screening then the mechanism of action can be further investigated in vitro or in animal studies.

Discover more at abcam.com/MitoSciences

Mitotox™ Solutions

Stages

- Screening
- Investigation

Samples

- In Vitro
- Ex Vivo

Assay Types

- ATP Detection
- Mitochondrial Potential
- Membrane Potential
- Mitochondrial Biogenesis
- Proapoptosis
- Oxygen Consumption
- Lactate Production
- mitochondrial enzyme activity and expression
- Mitochondrial membrane potential
- Mitochondria-protein expression
- Mitochondria-membrane integrity
- Mitochondria-nuclear translocation
- Inflammation
- Reactive Oxygen Species
- Lactate Production
- Oxygen Consumption
- Lactate Production

Product name Reactivity Product code
JC-1 Mitochondrial Membrane Potential Kit green ex cit, emi, red
ab117556
JC-1 Mitochondrial Membrane Potential Kit red ex cit, emi, red
ab117570
Luminescent ATP Detection Kit green ex cit, emi, red
ab117573
Mitobiogenesis™ In-Cell ELISA Kit hu, ms, rat, cow
ab117576
Mitobiogenesis™ In-Cell ELISA Kit (CytoGreen) hu, ms, rat, cow
ab117577
Mitobiogenesis™ WB Antibody Cocktail hu, ms, rat, cow
ab125300
PARP-1 (cleaved) In-Cell ELISA Kit hu, ms, rat, cow
ab117574
Luminescent ATP Detection Kit (Colorimetric) hu, ms, rat, cow
ab117571
Luminescent ATP Detection Kit (Colorimetric) hu, ms, rat, cow
ab117575
Luminescent ATP Detection Kit (Colorimetric) hu, ms, rat, cow
ab117578

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