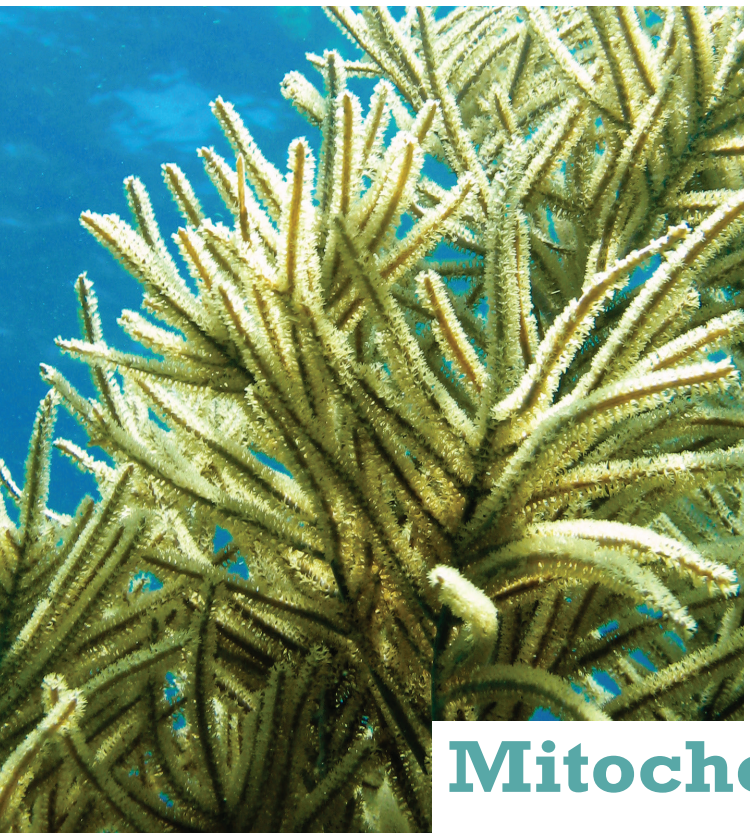
ISSUE 21

CAYMAN CURRENTS



Mitochondrial Health

IN THIS CURRENT:

Mitochondria and Cellular Homeostasis: Beyond ATP Synthesis

by David L. Hoffman, Ph.D.

Over the course of evolution, mitochondria have played essential roles in the continued development of higher organisms. Without mitochondria, it is questionable whether multicellular organisms would have evolved at all. Possessing their own DNA and transcription machinery, strong evidence supports that mitochondria were once free-living aerobic bacteria during the Statherian period. Within this period, α-proteobacteria became part of a multicellular system when engulfed by anaerobes. This relationship, described as symbiotic, proved to be mutually beneficial by providing a source of hydrogen, and a means for detoxifying oxygen, for the anaerobe in reciprocal exchange for a hospitable environment for the aerobe in which to thrive. Since then, mitochondria have become integrated into the crux of cellular function.[1] Critical for the maintenance of homeostasis, the mitochondrion functions as a source of raw materials for amino acid and heme biosynthesis, a buffering system for Ca^{2+},[2] a sensor for O_2, a gatekeeper for apoptotic signaling, a source of reactive oxygen species (ROS), and as heat source for certain vertebrates (brown adipose tissue). Their most important role, and the one for which they are best known, is the production of ATP through oxidative phosphorylation. It is through this role that mitochondria have shaped our physiology by facilitating the development of complex cardiovascular, digestive, and hepatic systems to efficiently transport O_2 and nutrients to cells and to remove waste generated through metabolic reactions. This article is an introduction to basic mitochondrial function and will touch on a few of the many important roles mitochondria play in cellular biology.

The mitochondrion is well known for its ability to efficiently convert metabolic byproducts into ATP. This conversion occurs by the electron transport chain (ETC) through the oxidation of reducing equivalents generated during glycolysis, the tricarboxylic acid (TCA) cycle and β-oxidation. The electron transport chain

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consists of four primary complexes (I-IV), which, through a series of redox reactions, facilitate the reduction of O_2 and the translocation of protons from the matrix to the intermembrane space. Since the inner mitochondrial membrane is impermeable, these translocated protons establish a gradient, or membrane potential ($\Delta\psi_M$), to be utilized by the ATP synthase. This proton gradient is essential for the synthesis of ATP and correlates directly with the rate of O_2 consumption (OCR) by the ETC. The relationship between the ETC and ATP synthesis is linked by $\Delta\psi_M$, which is described using the term "coupled". Compounds that dissipate the $\Delta\psi_M$, and as a result, increase OCR (*e.g.*, FCCP), are classified as uncouplers, whereas other compounds that dissipate $\Delta\psi_M$ by preventing OCR or the translocation of protons by the ETC, are classified as inhibitors. Both uncouplers and inhibitors can negatively affect the efficiency of the mitochondrion through the dissipation of $\Delta\psi_M$.

Mitochondrial uncoupling occurs naturally in brown adipose tissue (BAT), which derives its color from the excess of mitochondria. In mammals, BAT is known to induce non-shivering thermogenesis due to the expression of Uncoupling Protein 1 (UCP1), which uses $\Delta\psi_M$ to generate heat, resulting in high OCR, with little ATP production. Three types of UCPs, appropriately named 1, 2, and 3, have currently been identified. Whereas UCP1 is expressed only in BAT, the other two are expressed in a variety of tissue types.[3,4] UCPs function not only to generate heat, but also to regulate $\Delta\psi_M$. Activation of UCPs has been shown to correlate with oxidative stress and ROS. All UCPs are inhibited by GDP whereas genipin specifically inhibits UCP2. In addition to UCPs, mitochondria also possess a basal proton leak, which helps to prevent dielectric breakdown due to hyperpolarization. For more information on proton leak see publications from Martin Brand's group.[5,6]

While providing an energy intermediate to drive ATP synthesis, $\Delta\psi_M$ also influences the generation of ROS. To be more precise, higher $\Delta\psi_M$ results in decreased OCR, which in turn, leads to increased levels of ROS generation. The relationship between $\Delta\psi_M$ and ROS generation correlates to the effect of $\Delta\psi_M$ on OCR. Since OCR is proportional to the rate of electron transfer ($4e^-/O_2$), OCR dictates the redox status of the ETC. Because of this, a slower OCR results in a more reduced ETC, which is more likely to produce ROS at one of the ROS generating sites. These sites of ROS generation include (but are not limited to) complexes I, III, and the electron transport flavoprotein, which is involved in β-oxidation. The production of ROS by the ETC depends on both the concentration of electron donors ($R^\bullet$) and the concentration of electron acceptors (*e.g.*, O_2).[7] Under conditions where OCR is high (*e.g.,* actively phosphorylating mitochondria or in uncoupled mitochondria) the electron transport chain is more oxidized, therefore making it thermodynamically less favorable for ROS production to occur.[8-13] However, when OCR is low (*e.g.,* non-phosphorylating or in mitochondria with high $\Delta\psi_M$) and not limited by O_2, ROS generation is high, due to a more reduced ETC. Under conditions where O_2 is limiting (*e.g.,* hypoxia), the potential to generate ROS is high, yet, in isolated mitochondrial systems, generation of ROS does not increase due to a lack of an electron acceptor.[9] Paradoxically, a burst of mitochondrial ROS has been shown to occur under hypoxic conditions aiding in the stabilization of the hypoxia inducible factor 1 (HIF-1).[14]

The chemiosmotic proton gradient generated by the ETC is the driving force behind virtually all mitochondrial function. This $\Delta\psi_M$, which provides the driving force for ATP synthesis, heat generation, and ROS production, also allows mitochondria to function as cellular Ca^{2+} buffers. Using specialized Ca^{2+} transporters (Ca^{2+} uniporter and RaM), Ca^{2+} is transported into the mitochondrial matrix, along with water, resulting in swelling of the inner mitochondrial membrane.[15] The ability of mitochondria to buffer Ca^{2+} is critical for nominally functioning myocytes and neurons. However, a careful balance must be maintained. Should the mitochondria take up excess Ca^{2+} (as occurs during ischemia-reperfusion injury), the inner mitochondrial membrane will become permeable *via* opening of the mitochondrial permeability transition pore (mPTP). An open mPTP results in instantaneous mitochondrial depolarization, release of cytochrome *c*, and ultimately cell death. Opening of the mPTP can also

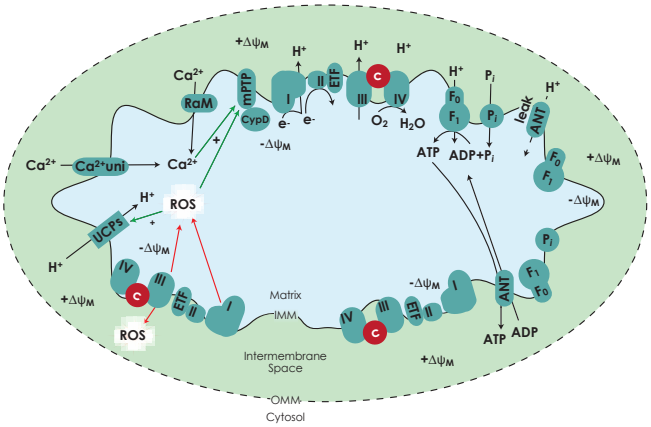

Figure 1- Illustration of basic mitochondrial functions outlined in the text. The electron transport chain is shown producing ROS and generating a proton gradient through the reduction of O_2. This is then utilized by the F_1F_0 ATP synthase (complex V) to generate ATP from ADP and P_i.The mitochondrial membrane potential is indicated by $\Delta\psi_M$ with + or – showing the respective charge. Abbreviations are as follows: IMM = inner mitochondrial membrane; OMM = outer mitochondrial membrane; ROS = reactive oxygen species; UCPs = uncoupling proteins; Ca^{2+}uni = Ca^{2+} uniporter; RaM = rapid mode of Ca^{2+} uptake; mPTP = mitochondrial permeablilty transition pore; CypD = cyclophilin D; P_i = phosphate, and its respective transporter; ANT = adenine nucleotide transporter

be triggered by oxidative stress. In small amounts, ROS generation can regulate $\Delta\psi_M$ by activating UCPs, whereas large amounts can overwhelm antioxidant defenses and result in the opening of mPTP. For a more detailed review on the balance between Ca^{2+} and ROS, see Brookes *et al.*[16]

This dynamic balance between Ca^{2+} and ROS sensitizes mitochondria to diseases affecting oxidant levels, glucose levels, and ion homeostasis. While many of these diseases are the focus of the pharmaceutical industry, some of the recent compounds developed to treat these diseases also have adverse effects on mitochondrial function. One such compound is the diabetes drug metformin, which inhibits complex I. Effects of other drugs range from inhibiting the ETC, inhibiting ATP synthase, or a mild to severe uncoupling, thus making the mitochondrion susceptible to drug induced toxicity.

While mitochondria are critical in powering a number of cellular processes, they are also uniquely adapted to aid the cell in functions that are independent of ATP synthesis. The recent edition of Bioenergetics 4 is a comprehensive resource for describing these detailed and complex mechanisms.[18] Within this edition of Cayman Currents, a number of reagents and kits are highlighted, each one targeted specifically towards a unique aspect of mitochondrial biochemistry. With further research, we can establish a better understanding of these unique organelles which are essential for maintaining biological homeostasis.

References

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Evaluating Mitochondrial Toxicity

A compound of interest is found to be cytotoxic through an unknown mechanism. Since mitochondrial toxicity can affect normal heart, liver, and brain function, we encourage you to figure out if it is indeed a mitigating factor. The process below walks you through how to evaulate mitochondrial toxicity with Cayman's line of assay kits.

Start by testing the compound with our Oxygen Consumption Rate Assay Kit (MitoXpress® -Xtra HS Method)

Review your data. Does the compound have an effect on oxygen consumption?

Yes

No

Interesting. Did oxygen consumption increase or decrease?

Increased

MITOTIP:
The ETC will increase oxygen consumption to compensate for small amounts of uncoupling. **Severe uncoupling, where membrane potential drops below 180 mV, will prevent ATP synthesis.**

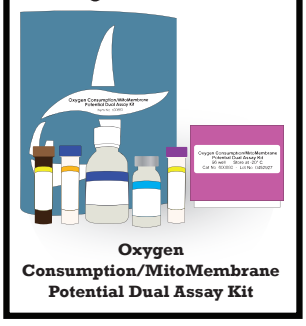
An increase in oxygen consumption **could be** a result of uncoupling mitochondria.

To find out, screen the compound using our **Oxygen Consumption/MitoMembrane Potential Dual Assay Kit** (w/uncouplers as positive controls).

Analyze your data. Is uncoupling present in the therapeutic range?

No

Yes



Oxygen Consumption/MitoMembrane Potential Dual Assay Kit

Decreased

A decrease in oxygen consumption could be the result of electron chain inhibition.

To find out, screen the compound with one of our **MitoCheck complex I, II, III, IV Activity Assay Kits**

mito✓

Analyze your data. Are any of the ETC complexes inhibited?

No

Yes

Is there full inhibition of complex I and II, III, or IV within the therapeutic range?

No

Yes

MITOTIP:
Electrons enter at complexes I and II before they move on to complexes III and IV. **Inhibition of complex I is linked to neurotoxicity,** which causes long term effects harmful to the human body.

Is complex I inhibited within the therapeutic range?

No

Yes

The compound is affecting another aspect of metabolic function.

The compound is mildly toxic and likely to exhibit toxic effects related to mitochondrial dysfunction.

The compound is acutely toxic to mitochondria.

Congratulations!
The compound likely has minimal effects on mitochondrial function. You're free to move on with your research.

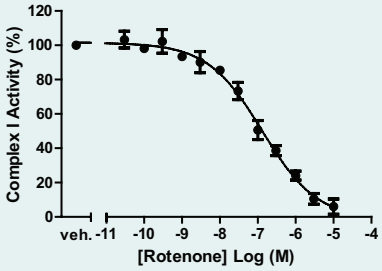
MitoCheck ETC Activity Assays

• Isolated bovine heart mitochondria provided in each kit • No need to preincubate with antibodies • Amenable to high throughput

MitoCheck Complex I Activity Assay Kit 700930

Summary: Complex I (NADH oxidase/Co-enzyme Q reductase) is one of the major sites of electron entry into the mitochondrial ETC. Complex I catalyzes the 2 electron oxidation of NADH followed by the reduction of ubiquinone (Q) to form ubiquinol (QH_2) and ultimately the reduction of the terminal electron acceptor, O_2. During the passage of electrons from NADH to Q, the translocation of four protons (H^+) from the mitochondrial matrix to the intermembrane space occurs, contributing to the chemiosmotic proton gradient, which is required for oxidative phosphorylation. Cayman's MitoCheck Complex I Activity Assay allows for the activity of complex I to be determined without the need to isolate mitochondria or pre-incubate with antibodies. The rate of NADH oxidation is measured by a decrease in absorbance at 340 nm and is proportional to the activity of complex I.

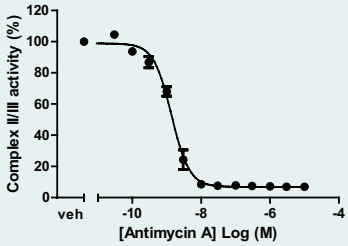
96 wells



MitoCheck Complex II/III Activity Assay Kit 700950

Summary: Complex III (CoQ cytochrome *c* oxidoreductase) is an essential protein for mitochondrial oxidative phosphorylation. Complex III functions as both a gatekeeper for mitochondrial respiration and as a major source of reactive oxygen species III. It accepts electrons from complexes I and II in the form of QH_2, the reduced form of the electron carrier ubiquinone. Once bound to complex III, QH_2 undergoes a series of redox reactions, known as the Q-cycle. During the Q-cycle, electrons are passed from QH_2 onto cytochrome *c via* the Reiske iron-sulfur protein and cytochrome c_1 resulting in the translocation of $4H^+$ and the generation of $O_2^{\bullet-}$. Cayman's MitoCheck Complex II/III Activity Assay measures the reduction of excess cytochrome *c* (550 nm absorbance) as catalyzed by complex III. This assay is coupled to succinate co-enzyme Q oxidoreductase (complex II) for the generation of QH_2. Due to the dependence on complex II activity, a counterscreen for complex II activity (Item No. 700940) should be performed in order to truly measure complex III activity.

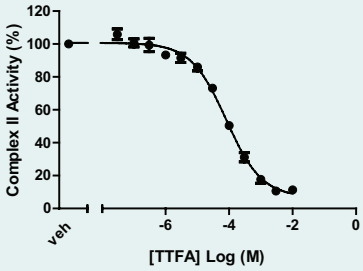
96 wells



MitoCheck Complex II Activity Assay Kit 700940

Summary: Complex II (succinate dehydrogenase/co-enzyme Q reductase) is one of the major sites of electron entry into the mitochondrial ETC. Complex II catalyzes the oxidation of succinate to fumarate and in the process reduces ubiquinone (Q) to ubiquinol (QH_2). Ultimately, oxidation of succinate will lead to reduction of O_2, the terminal step in mitochondrial respiration. Cayman's MitoCheck Complex II Activity Assay allows for the activity of complex II to be determined without the need to isolate mitochondria or pre-incubate with antibodies. As complex II oxidizes succinate, electrons are passed to an analog of ubiquinone and then on to DCPIP, which, when oxidized, absorbs in the 600 nm range. The absorbance of DCPIP will decrease upon reduction. Thus, complex II activity is measured as a decrease in absorbance at 600 nm over time.

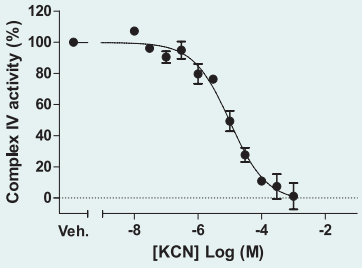
96 wells



MitoCheck Complex IV Activity Assay Kit 700990

Summary: Complex IV (cytochrome *c* oxidase) is the terminal electron acceptor in the mitochondrial electron transport chain. Complex IV functions by oxidizing cytochrome *c* and completing a four electron reduction of O_2 to form water. During this process, two H^+ are translocated from the mitochondrial matrix to the intermembrane space, contributing the mitochondrial membrane potential required for ATP synthesis. Common inhibitors of complex IV include potassium cyanide, azide, O_2 limitation, nitric oxide, and carbon monoxide. Cayman's MitoCheck Complex IV Activity Assay is designed to measure the direct oxidation of cytochrome *c* by complex IV, in an isolated bovine heart mitochondrial system that is supplied within the kit.

96 wells



ask Cayman

QUESTIONS FROM THE FIELD

How do the electron transport chain kits work without using antibodies?

Cayman's kits take advantage of specific inhibitors to shut down the activity of the ETC complexes not being targeted by each assay. This eliminates the need to isolate the specific ETC complexes prior to measurement of their activity.

Oxygen Consumption Rate Assays - in partnership with LUXCEL BIOSCIENCES

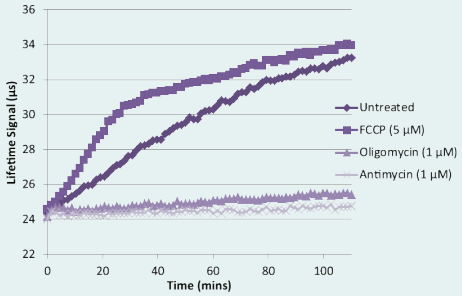
The oxygen consumption rate of cells is an important indicator of normal cellular function. It is used as a parameter to study mitochondrial function and as a marker of factors triggering the switch from healthy oxidative phosphorylation to aerobic glycolysis in cancer cells. Oxygen consumption is traditionally measured using an oxygen electrode, a specialized piece of equipment that has low sample throughput. The phosphorescent oxygen probe, MitoXpress®Xtra, developed by Luxcel Biosciences, offers a novel method for analyzing oxygen consumption in whole cells. Cayman's Cell-Based Oxygen Consumption Rate Assay Kits utilize this probe to measure oxygen consumption rate in living cells in a 96-well plate format. The kits include a mitochondrial electron transport chain inhibitor for use as a positive control and an oxygen depletion enzyme for use as a reference. Two of these novel kits combine the probe with additional readouts to offer a multiplex assessment of mitochondrial performance.

Oxygen Consumption Rate Assay Kit (MitoXpress® - Xtra HS Method) 600800

OCR

Summary: The OCR of cells is an important indicator of normal cellular function. It is used as a parameter to study mitochondrial function and as a marker of factors triggering the switch from healthy oxidative phosphorylation to aerobic glycolysis in cancer cells. Cayman's cell-based OCR Assay Kit (MitoXpress® - Xtra HS Method) utilizes the phosphorescent oxygen probe, MitoXpress® - Xtra to measure OCR in living cells. Antimycin A, an inhibitor of the mitochondrial electron transport chain, is included to be used as a positive control. Glucose oxidase is also included in the kit to be used as a reference for oxygen depletion.

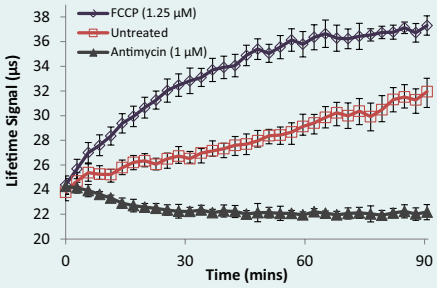
96 wells



Oxygen Consumption/MitoMembrane Potential Dual Assay Kit 600880

Summary: Cayman's Oxygen Consumption/Mitochondrial Membrane Potential Dual Assay Kit is a multiplex assay designed to provide a comprehensive picture of mitochondrial performance in living cells. This assay simultaneously measures both oxygen consumption rate and mitochondrial membrane potential by utilizing MitoXpress® - Xtra, a phosphorescent oxygen probe, and the cationic dye, JC-1. Antimycin A, an inhibitor of the mitochondrial electron transport chain, is included as a control. Additionally, glucose oxidase is provided as a reference for oxygen depletion.

96 wells



Typical lifetime profiles of MitoXpress® - Xtra measuring the effect of mitochondrial inhibition (Antimycin treatment) and uncoupling (FCCP treatment) on cell respiration. Measurement made immediately post treatment.

Oxygen Consumption/Glycolysis Dual Assay Kit 601060

Summary: Cayman's Oxygen Consumption/Glycolysis Dual Assay Kit is a multi-parameter approach to measure cellular oxygen consumption and glycolysis in living cells. This assay utilizes MitoXpress® - Xtra, a phosphorescent oxygen probe, to measure oxygen consumption rate, and also quantifies extracellular lactate as a readout for glycolysis. Antimycin A, an inhibitor of the mitochondrial electron transport chain, is included as a control. The kit can be used for efficient screening of compounds that modulate mitochondrial and glycolytic function in cultured cells.

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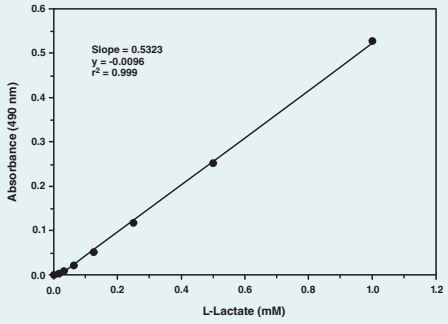


ask Cayman

QUESTIONS FROM THE FIELD

Can you determine mitochondrial toxicity by measuring just cellular oxygen consumption?

Oxygen is used by many enzymes throughout the cell, not just mitochondria. Therefore, cellular oxygen consumption simply provides a whole-cell view of how fast oxygen is being consumed. Looking at mitochondrial activity is more focused. With the MitoCheck ETC Activity Assay Kits, the activity of specific subunits of the electron transport chain can be measured and potential effects of a compound on the complex can be determined.

Mitochondrial Inhibitors

Electron Transport Chain Inhibitors			
Item No.	Item Name	Key Information	Sizes
11898	Atpenin A5	Selectively inhibits complex II respiratory enzymes (IC_{50}s = 12 and 3.7 nM in nematode and mammalian mitochondria, respectively)	250 µg • 1 mg
15218	FCCP	Uncouples oxidative phosphorylation in mitochondria, disrupting ATP synthesis by transporting protons across cell membranes	10 mg • 50 mg
15159	HQNO	Blocks mitochondrial complexes I and III; inhibits the activity of the NADH oxidase; inhibits NADH:ubiquinone-1 oxidoreductase activity; blocks proton channels needed to reduce oxygen	5 mg • 10 mg • 50 mg
13118	Metformin (hydrochloride)	A biguanide derivative that inhibits complex I of the mitochondrial respiratory-chain	1 g • 5 g
15379	Piericidin A	Irreversible inhibitor of mitochondrial complex I that strongly associates with ubiquinone binding sites in both mitochondrial and bacterial forms of the enzyme	2 mg • 10 mg
13995	Rotenone	Inhibits NAD-linked substrate oxidation of NADH dehydrogenase (EC_{50} = 10 pM), inhibiting the transfer of electrons from iron-sulfur centers in complex I to ubiquinone	1 g • 5 g • 10 g • 25 g

ATP Synthase Inhibitors			
Item No.	Item Name	Key Information	Sizes
11342	Oligomycin A	Inhibits the mitochondrial F_1F_0-ATP synthase	1 mg • 5 mg • 10 mg
11343	Oligomycin B	A nonselective inhibitor of ATP synthase	1 mg • 5 mg • 10 mg
11341	Oligomycin Complex	A mixture of Oligomycins A, B, and C	5 mg • 10 mg
15377	Venturicidin A	A macrolide antibiotic that inhibits bacterial and mitochondrial ATP synthases	1 mg • 5 mg

Additional Inhibitors			
Item No.	Item Name	Key Information	Sizes
15611	CGP 37157	A selective inhibitor of the mitochondrial sodium-calcium exchanger (IC_{50} = 0.36 µM in isolated mitochondria)	10 mg • 50 mg
10010622	Genipin	Inhibits UCP2 activity	5 mg • 10 mg 25 mg • 50 mg
15559	Mdivi 1	Selectively inhibits mitochondrial division by blocking dynamin GTPase activity in yeast (IC_{50} = 1-10 µM) and mammalian cells (IC_{50} ~50 µM); prevents mitochondrial outer membrane permeabilization	5 mg • 10 mg 25 mg • 50 mg

Substrates			
Item No.	Item Name	Key Information	Sizes
9000939	L-Arachidonoylcarnitine chloride	An acylcarnitine formed from carnitine conjugated to arachidonic acid that may be useful as a marker of mitochondrial function	1 mg • 5 mg 10 mg
15475	Idebenone	A natural quinone that serves as a cofactor in the mitochondrial electron transport chain	10 mg • 25 mg 50 mg • 100 mg
9001873	L-Propionylcarnitine chloride	A carnitine derivative formed by carnitine acetyltransferase during β-oxidation of uneven chain fatty acids that plays a role in mitochondrial metabolism	25 mg • 50 mg 100 mg

Nampt

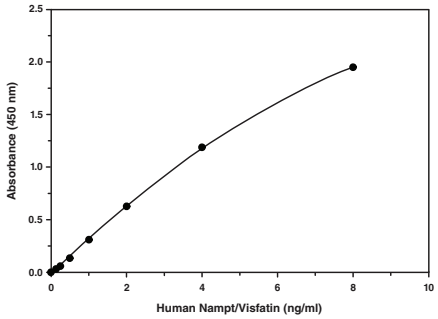
Nampt/Visfatin (human) EIA Kit 579020

Nicotinamide Phophoribosyltransferase/Visfatin

Limit of Detection: 30 pg/ml

Summary: Nampt is the rate-limiting enzyme in the salvage pathway for the biosynthesis of NAD^+ from nicotinamide. It has important roles, both intra- and extracellularly, in cellular metabolism and disease. Extracellularly, the levels of Nampt in serum correlate with body mass index and body fat mass, are increased during inflammation, and are decreased with liver cirrhosis. Extracellular Nampt regulates insulin secretion in β cells by regulating systemic NAD^+ biosynthesis. Nampt levels and expression in serum, circulating leukocytes, and tissues may be useful biomarkers for inflammation, cancer, obesity, and other diseases. Cayman's Nampt/Visfatin (human) EIA Kit is an immunometric assay that can be used to measure Nampt/Visfatin in human serum.

96 wells



•Also Available: **Nampt/Visfatin (mouse/rat) EIA Kit** (579040)

NAD+/NADH Cell-Based Assay Kit 600480

Nicotinamide adenine dinucleotide/Nicotinamide adenine dinucleotide, reduced

Summary: NAD exists in an oxidized form, NAD^+, as well as a reduced form, NADH. NAD^+, the main free form in cells, functions in modulating cellular redox status and by controlling signaling and transcriptional events, making it and related enzymes drug targets for various metabolic disorders. Cayman's NAD^+/NADH Cell-Based Assay Kit provides a colorimetric method for measuring intracellular NAD^+/NADH in cultured cells.

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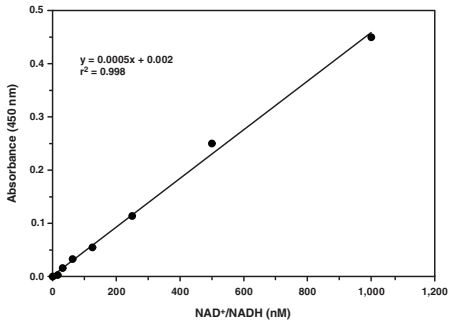


Nampt Inhibitors			
Item No.	Item Name	Key Information	Sizes
13670	CAY10618	A potent inhibitor of Nampt (IC_{50} = 3.0 nM)	500 µg • 1 mg • 5 mg • 10 mg
13287	FK-866	A highly specific, non-competitive inhibitor of Nampt (K_i = 0.4 nM), causing gradual NAD^+ depletion	5 mg • 10 mg • 25 mg • 50 mg

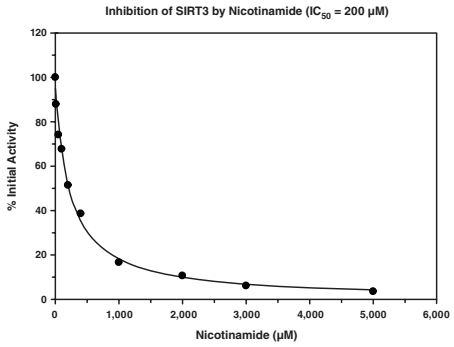
Nampt Antibody			
Item No.	Item Name	Application(s)	Specificity
10813	Nampt Monoclonal Antibody (Clone OMNI 379)	ELISA, FC, ICC, IHC, IP, WB	(+) human, mouse, rat

Sirtuins

SIRT3 Direct Fluorescent Screening Assay Kit 10011566

Summary: SIRT3 is a mitochondrial protein that plays a role in metabolic regulation and other cellular processes. It is the only human sirtuin with a direct genetic link with longevity and has been implicated in metabolic dysfunction, aging, cancer, and neurodegenerative disease. Cayman's SIRT3 Direct Fluorescent Screening Assay Kit provides a convenient fluorescence-based method for screening SIRT3 inhibitors or activators.

96 wells



Sirtuin Proteins			
Item No.	Item Name	Key Information	Sizes
10011194	SIRT3 (human recombinant)	Source: active recombinant N-terminal His-tagged enzyme amino acids 101-399 purified from *E. coli* • M_r: 37 kDa	25 µg • 50 µg 100 µg
10317	SIRT4 (human recombinant)	Source: recombinant N-terminal GST-tagged enzyme expressed in *E. coli* • M_r: 61.9 kDa	25 µg • 50 µg 100 µg
10318	SIRT5 (human recombinant)	Source: recombinant N-terminal GST-tagged enzyme expressed in *E. coli* • M_r: 60.6 kDa (GST-tagged); 26 kDa (native)	25 µg • 50 µg 100 µg

Sirtuin Antibody			
Item No.	Item Name	Application(s)	Specificity
13476	SIRT5 Polyclonal Antibody	WB	(+) human, mouse, rat

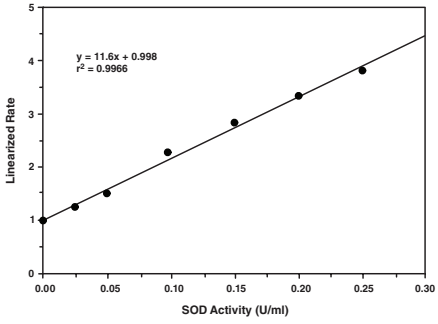
Superoxide Dismutase

Superoxide Dismutase Assay Kit 706002

SOD

Summary: Significant amounts of SOD in cellular and extracellular environments are crucial for the prevention of diseases linked to oxidative stress. The reaction catalyzed by SOD is extremely fast, having a turnover of 2 x 10^6 $M^{-1}sec^{-1}$ and the presence of sufficient amounts of the enzyme in cells and tissues typically keeps the concentration of superoxide very low. Quantification of SOD activity is therefore essential in order to fully characterize the antioxidant capabilities of a biological system. Cayman's SOD Assay kit is a fast and reliable assay for the measurement of SOD activity from plasma, serum, tissue homogenates, and cell lysates. Cu/Zn-, Mn-, and Fe-SOD activity is assessed by measuring the dismutation of superoxide radicals generated by xanthine oxidase and hypoxanthine in a convenient 96-well format.

96 wells
480 wells



ask Cayman
QUESTIONS FROM THE FIELD

Why study toxicity in the mitochondria?

Toxicity in the mitochondria has been linked to many diseases ranging from neurodegenerative conditions to epilepsy, diabetes, and cancer. The impact mitochondrial dysfunction has on so many diseases makes it an important field of basic study to help understand therapeutic opportunities. Also, mitochondrial toxicity is often an undesirable outcome in the drug development process, causing unwanted side effects. Therefore, testing for mitochondrial toxicity is essential to providing safer drugs in the future.

SOD Antibodies			
Item No.	Item Name	Application(s)	Specificity
10011388	Cu/Zn SOD (human) Polyclonal Antibody	EIA, IHC, IP, WB	(+) human, mouse, bovine, monkey, coral, canine, hamster, porcine, rabbit, ovine, and rat
10011390	Mn SOD (human) Polyclonal Antibody	IHC, IP, WB	(+) human, rat, mouse, bovine, canine, chicken, gerbil, guinea pig, porcine, hamster, monkey, rabbit, ovine, and *xenopus*
10011389	Mn SOD (rat) Polyclonal Antibody	ELISA, IHC, IP, WB	(+) human, rat, mouse, bovine, canine, chicken, *Drosophila*, guinea pig, porcine, hamster, monkey, rabbit, ovine, and *xenopus*

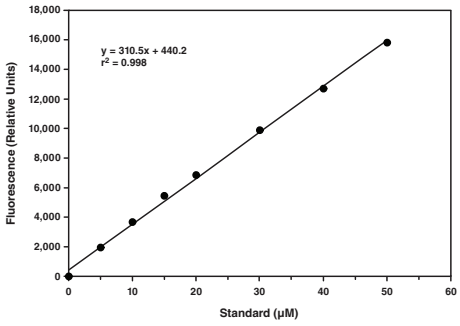
Additional Assays

Aconitase Fluorometric Assay Kit 700600

Limit of Detection: 0.15 nmol/min/ml

Summary: Cayman's Fluorometric Aconitase Activity Assay provides a fluorescence-based method for detecting aconitase activity from tissue homogenates or cell lysates. In this assay, citrate is isomerized by aconitase into isocitrate, which is then converted to α-ketoglutarate in a reaction catalyzed by isocitrate dehydrogenase. These reactions are monitored by measuring the formation of NADPH in a reaction with a substrate that yields a highly fluorescent product.

96 wells

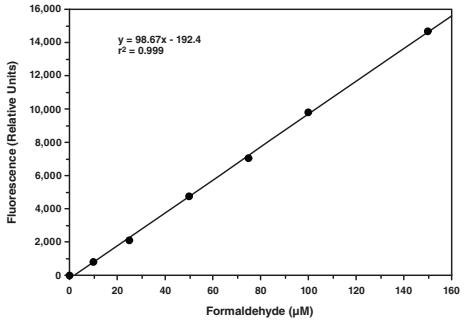


Creatine Kinase Fluorometric Assay Kit 700630

CK

Summary: CK catalyzes the reversible phosphorylation of creatine by ATP to form ADP and phosphocreatine, the major storage form of high energy phosphate in muscle. CK (in blood) is assayed as a marker of myocardial infarction (heart attack), rhabdomyolysis (severe muscle breakdown), muscular dystrophy, and acute renal failure. Cayman's CK Fluorometric Assay provides a convenient method for detecting total CK activity in plasma and serum.

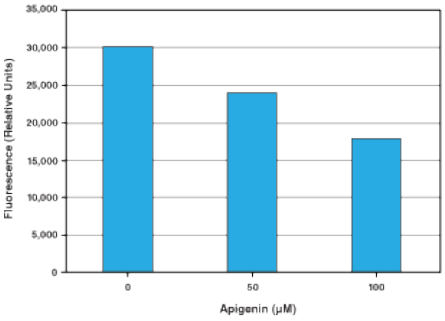
96 wells



Glucose Uptake Cell-Based Assay Kit 600470

Summary: The rate of glucose uptake in cells is dynamic and tightly regulated to maintain normal cell homeostasis. Cancer cells exhibit increased glucose uptake and metabolism by aerobic glycolysis in order to support a high rate of proliferation. Cayman's Glucose Uptake Cell-based Assay Kit provides a convenient tool for studying modulators of cellular glucose uptake. The kit employs 2-NBDG, a fluorescently-labeled deoxyglucose analog, as a probe for the detection of glucose taken up by cultured cells. Apigenin, a flavonoid that has been reported to be an inhibitor of glucose transport, is included as a control.

1 ea

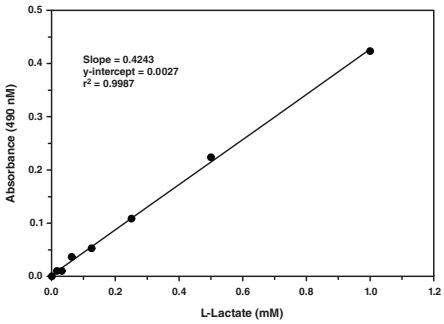


Measurement of 2-NBDG uptake in LS-180 cells grown in glucose- and serum-free medium and treated with various doses of Apigenin.

Glycolysis Cell-Based Assay Kit 600450

Summary: Cayman's Glycolysis Cell-Based Assay Kit provides a colorimetric method for detecting extracellular L-lactate, the end product of glycolysis, in cultured cells. In the assay, lactate dehydrogenase catalyzes the oxidation of lactate to pyruvate, in which the formed NADH reduces a tetrazolium substrate (INT) to a highly-colored formazan which absorbs strongly at 490-520 nm. The amount of formazan produced is proportional to the amount of lactate released into the culture medium and can be used as an indicator of the cellular glycolytic rate. This non-invasive sampling format leaves the cells intact and, thus, allows multiplex testing for additional markers.

1 ea

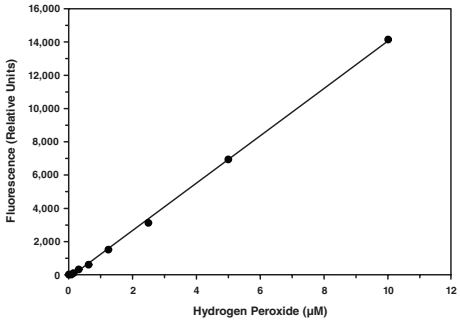


Hydrogen Peroxide Cell-Based Assay Kit 600050

H_2O_2

Summary: H_2O_2, a natural by-product of oxygen metabolism, has strong oxidizing capacity and is thus considered a highly reactive oxygen species. It is well established that H_2O_2 is a cytotoxic agent, but evidence also suggests that H_2O_2 may be an important regulator of eukaryotic signal transduction. Cayman's H_2O_2 Cell-Based Assay Kit provides a simple fluorometric method for the sensitive quantitation of H_2O_2 in cultured cells.

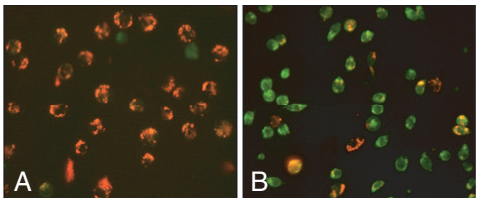
96 wells
480 wells



JC-1 Mitochondrial Membrane Potential Assay Kit 10009172

Summary: Mitochondrial membrane potential, $\Delta\psi_M$, is used as an indicator of cell health. JC-1 is a cationic dye that selectively enters into mitochondria and reversibly changes color from green to red as the membrane potential increases. In healthy cells with high mitochondrial $\Delta\psi_M$, JC-1 spontaneously forms complexes known as J-aggregates with intense red fluorescence. In apoptotic or unhealthy cells with low $\Delta\psi_M$, JC-1 remains in the monomeric form, which shows only green fluorescence.

100 tests



Effect of staurosporine on mitochondrial potential in Jurkat cells. *Panel A*: untreated cells show most cells had strong J-aggregation (red). *Panel B*: staurosporine-treated cells show a majority of the cells stained green due to low $\Delta\psi_M$.

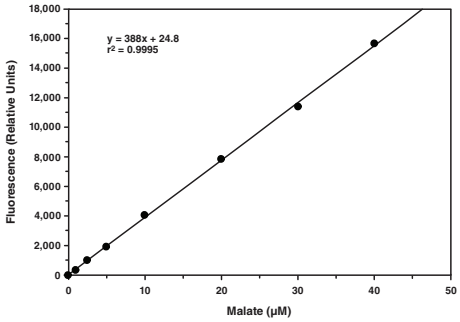
Mitochondrial Transmembrane Potential

Item No.	Item Name	Key Information	Sizes
15003	JC-1	Cationic dye used to study mitochondrial integrity in the context of cellular apoptosis; changes fluorescence characteristics with alteration in mitochondrial transmembrane potential ($\Delta\Psi_M$)	1 mg • 5 mg • 10 mg

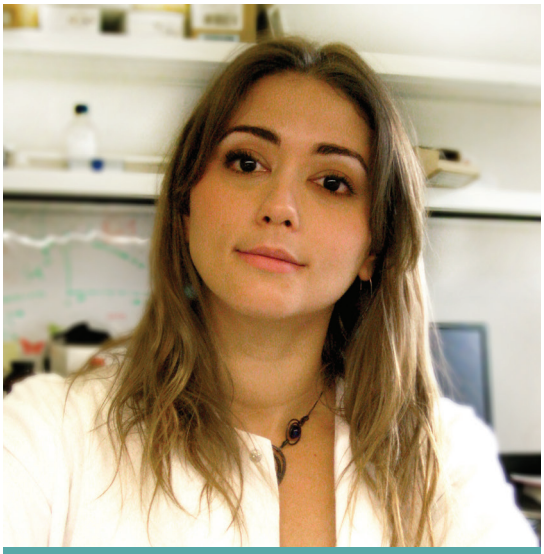
Malate Fluorometric Assay Kit 700790

Summary: L-Malate, the anionic form of L-malic acid, is an intermediate in the TCA cycle. It is a source of CO_2 in plants, improves muscle performance post-exercise, and can act as a metal chelator. Cayman's Malate Fluorometric Assay provides a fluorescence-based method for detecting malate from plasma, serum, urine, tissue homogenates, and cell culture samples.

96 wells



Researcher Spotlight



Renata Goncalves, Ph.D.

Postdoctoral Research Scholar

Buck Institute for Research on Aging in the laboratory of Dr. Martin Brand

Where did you earn your Ph.D.? In what field?

I earned my Ph.D. in Biochemistry and Molecular Biology at the Instituto de Bioquímica Médica at the Federal University of Rio de Janeiro (Brazil) under the supervision of Dr. Marcus F. Oliveira.

What attracted you to Dr. Brand's lab at the Buck Institute for Research on Aging? What do you enjoy most about working in the lab?

My interest in Martin's research developed alongside my interest in mitochondrial metabolism. When I was a senior in high school, I applied for a scientific initiation program and was able to work in a molecular entomology laboratory at the Federal University of Rio de Janeiro. My project was to build an apparatus that would allow me to measure the respiration of large insects. This was the catalyst for my interest in bioenergetics! At seventeen, I started working in a lab at the Federal University where I would eventually complete my Ph.D. This interest in bioenergetics intensified when I met my Ph.D. advisor. He is a very intelligent man who offered me an interesting and challenging project that involved mosquito flight muscle mitochondria. We had to start from scratch and describe our own protocols. The first paper I read to research this task was from Martin's group. Since then, I have built an admiration for and interest in his work. It was obvious to me that if I wanted to understand mitochondrial metabolism in depth, I should work in his lab. When I contacted Martin, I was in the middle of my Ph.D. and envisioned being a postdoc in his lab. When I wrote to Martin, I was working at the NIH-NIAID with Dr. Barillas-Mury. I was asked to visit the Buck Institute to present my findings and told him that I would like to join his group for a postdoc. I am very happy to be here today. Martin is an exponent in the field and a great mentor to lean on for guidance. What I think is unique about working with him is his knowledge, especially in mitochondrial metabolism, and also his involvement in the projects and how he is available to talk about the results. Usually our lab has lunch together, and this is another important time to share ideas and thoughts in a more informal way.

What can you tell us about your current research project?

Our lab studies the mechanisms of reactive oxygen species (ROS) generation in mitochondria. Mitochondria are central to energy metabolism, and ROS production is intrinsic to oxidative phosphorylation. Mitochondrial ROS have been implicated in the genesis of many diseases including cancer and neurodegeneration. So far, we know that at least 10 sites are able to produce ROS in mitochondria isolated from skeletal muscle; therefore, ROS production should not be oversimplified as a single process. We want to characterize which sites are active under physiological and pathological conditions. Since each site is unique regarding its maximal rate of ROS production, it is crucial to understand what controls ROS formation and from which sites they are produced *in vivo*. Although it is well accepted that mitochondrial ROS production increases in many pathological states, we do not know if it is a result of an overall ROS increase or if specific sites misbehave. Our goal is to map all sites able to produce ROS in mitochondria and define their real rates *in vivo*. I want to characterize the contribution of each individual site under physiological conditions to be able to detect the sites that start to produce more ROS under pathological conditions. A few years ago it would have been unthinkable to normalize the misbehaved sites by using classical inhibitors of the electron transport system. These inhibitors would not only decrease ROS production but would also block electron flow, impacting oxygen consumption and ATP synthesis. However, our group has identified small compounds that specifically decrease ROS production from individual sites without impacting other mitochondrial functions. With this fabulous tool in hand, we can now modulate the ROS production from individual sites without changing important mitochondrial functions. My current project is to characterize the sites active when we incubate mitochondria in a semi-physiological medium mimicking muscle cells under rest or exercise conditions. We were able to measure the total ROS generated when mitochondria oxidized a complex mixture of substrates and identify which sites were responsible for the total ROS produced. Although it is a consensus that radical species are increased during exercise, my results show that mitochondrial ROS production is likely to be decreased. Importantly, the sites active during "rest" and "exercise" are not the same. We hypothesize that a similar scenario may operate under pathological conditions where specific sites may misbehave. Our ultimate goal will be to use the site-selective inhibitors we identified to reduce ROS formation specifically from misbehaved sites.

What are the next steps in your career? What do you plan/hope to do in the future?

My goal is to become a PI at a good university where I can teach and conduct research. I really like the Bay Area in San Francisco, and I envision myself in the future working at Berkeley or UCSF. I am preparing myself for this possibility, and I know to accomplish this goal that I have to be as productive as I can be, develop my own ideas, and publish in top quality journals. Also, I want to have a solid and consistent postdoc, which is my priority at the moment. That's why I chose to work in the lab of Dr. Brand.

What piece of advice do you have for fellow postdocs/researchers?

Be passionate about what you do. Add energy in your actions and thoughts. Always try to improve and be a better person in all senses. Listen carefully to the critiques that you receive, but don't let them push you down; use them to grow and improve.

Researcher Spotlight

Want to have your research featured in the Cayman Currents? Send a brief background to **marketing@caymanchem.com**

On Our Cover

Cayman's Current cover features a picture of a mantis shrimp, a stomatopod crustacean found in shallow tropical waters. Their bright colored exterior reminded us of the fluorescent stains used to evaluate cell health with our our assay kits. After learning the mantis shrimp had highly sensitive characteristics and a powerful punch, we knew it was the perfect symbol to call attention to the features of our mitochondrial health product line.

3 INTERESTING FACTS YOU MAY NOT HAVE KNOWN ABOUT THE MANTIS SHRIMP:

1. Mantis shrimp have the fastest punch in the world, delivering a blinding 500 Newton (112 lbs) blow to its prey at 23 m/s from a standing start.

2. Mantis shrimp have the most complex eyes in the animal kingdom with vision so precise they can see both polarized light and multispectral details. Their specialized ommatidia have at least 16 different photoreceptor types (12 for color sensitivity and others for color filtering) and are arranged so that each eye possesses trinocular vision.

3. Mantis shrimp have a tough exterior. It's so tough that researchers are studying its cell structure to try to create a new form of body armor for soldiers.

Q&A with David L. Hoffman, Ph.D.

Why do YOU find mitochondrial research interesting & exciting?

Mitochondria are very dynamic and complex organelles. This complexity wasn't something I truly appreciated until I started doing mitochondrial research. Bioenergetics, to me, isn't just about ATP production, but rather a study of the intricate biochemistry that needs to occur in order for that to take place; a lot of which takes place in the electron transport chain. The electron transport chain , in addition to generating the energy intermediate required for ATP synthesis (and calcium buffering, in which the mitochondrion plays an integral role), also functions as a sensor for intracellular oxygen, producing ROS for cell signaling. The mitochondrion possesses its own DNA, ribosomes, protein import and export mechanisms, its own transporters and its own protease. In addition to this, the mitochondria function not just as individual organelles, but as a network that is constantly undergoing fusion and fission. The status of this network is often indicative of cellular health, which is not surprising giving the integral role of mitochondria in maintaining cellular homeostasis. Because of its unique biochemistry and multifunctional role, I find it very difficult not to get excited about mitochondrial research!

What do you see as the future of mitochondrial research?

I think we've only scratched the surface. In the 10 years following Mitchell and Moyle's paper detailing chemiosmosis, interest in mitochondrial research really took off, then gradually began to fade. In fact, it was speculated that we had learned all there was to know about these organelles. However, with the 1994 discovery of cytochrome *c*'s role in apoptosis, mitochondrial research again took center stage! Now with major advances in technology, researchers are discovering new roles for mitochondria in cancer, diabetes, heart disease, and autoimmune diseases. While these advances in technology are allowing us to learn more about mitochondria, they only seem to result in more questions about how these organelles function and how they are integrated into our cellular makeup. Therefore, I believe that mitochondrial research is going to be around for a long, long time.

Isolated Mitochondria *vs.* Mitochondria in Cells

Which system is preferred when evaluating different aspects of mitochondrial function?

	Isolated Mitochondria	Cellular Mitochondria
Correlate oxygen consumption with electron transport chain (ETC) activity	✓	Oxygen consumption correlates with ETC in both isolated and cellular mitochondria, but there may be other sources of oxygen consumption in cellular mitochondria
Can measure respiratory acceptor control ratio (RCR)	✓	RCR can be measured in fresh isolated mitochondria or cellular mitochondria, but the measurement in cellular mitochondria is not as accurate since ADP is always present
Assay individual ETC enzymes	✓	Assaying individual ETC enzymes is not possible in cellular mitochondria
Measure membrane potential	✓	Membrane potential can be measured in both isolated and cellular mitochondria, but there is a greater chance of cell membrane interference in cellular mitochondria
Measure glycolytic function (lactate)	Measuring glycolytic function is not possible in isolated mitochondria because glycolysis occurs in the cytoplasm	✓

Helping Make Research Possible

1180 E. Ellsworth Road
Ann Arbor, MI 48108
www.caymanchem.com

Contact Us

EMAIL:
Sales sales@caymanchem.com
Customer Service custserv@caymanchem.com

PHONE: 800.364.9897 (USA and Canada only)
734.971.3335 (direct)
FAX: 734.971.3640
WEB: www.caymanchem.com

TECHNICAL SUPPORT

EMAIL: techserv@caymanchem.com
PHONE: 888.526.5351 (USA and Canada only)
734.975.3888 (direct)
WEB: www.caymanchem.com/techserv