

## BIOENERGETICS



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## 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,  $\alpha$ -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.<sup>1</sup> 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  $\text{Ca}^{2+}$ , a sensor for  $\text{O}_2$ , a gatekeeper for apoptotic signaling, a source of reactive oxygen species (ROS), and a heat source for certain vertebrates (brown adipose tissue; BAT).<sup>2</sup> 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  $\text{O}_2$  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  $\beta$ -oxidation. The ETC consists of four primary complexes (I-IV), which, through a series of redox reactions, facilitate the reduction of  $\text{O}_2$  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

► continue to page 2

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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 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 (UCP) 1, 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.<sup>3,4</sup> 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 guanosine diphosphate (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.<sup>5,6</sup>

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  $\beta$ -oxidation. The production of ROS by the ETC depends on both the concentration of electron donors ( $R^*$ ) and the concentration of electron acceptors (e.g.,  $O_2$ ).<sup>7</sup> Under conditions where OCR is high (e.g., actively phosphorylating mitochondria or in uncoupled mitochondria) the ETC is more oxidized, therefore making it thermodynamically less favorable for ROS production to occur.<sup>8-13</sup> 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.<sup>9</sup> 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).<sup>14</sup>

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 [ $Ca^{2+}$  uni] and rapid mode of  $Ca^{2+}$  uptake [RaM]),  $Ca^{2+}$  is transported into the mitochondrial matrix, along with water, resulting in swelling of the inner mitochondrial membrane.<sup>15</sup> 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

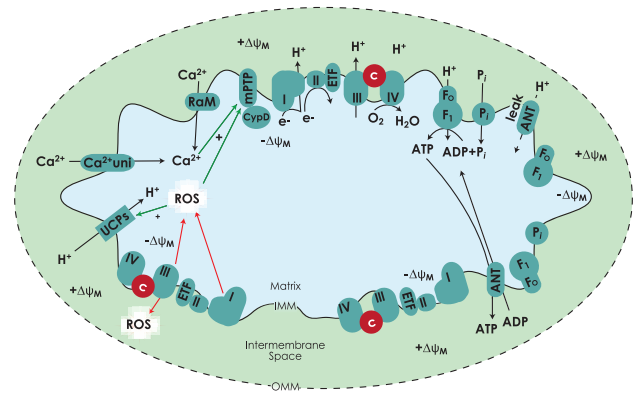


Illustration of basic mitochondrial functions outlined in the text. The ETC is shown producing ROS and generating a proton gradient through the reduction of  $O_2$ . This is then utilized by the  $F_1F_0$  ATP synthase (complex V), to generate ATP from ADP and  $P_i$ . The mitochondrial membrane potential is indicated by  $\Delta\psi_M$  with + or - showing the respective charge.

IMM inner mitochondrial membrane • OMM outer mitochondrial membrane • CypD cyclophilin D  $P_i$  phosphate, and its respective transporter • ANT adenine nucleotide translocase

mPTP results in instantaneous mitochondrial depolarization, release of cytochrome c, and ultimately cell death. Opening of the mPTP can also 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.*<sup>16</sup>

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.

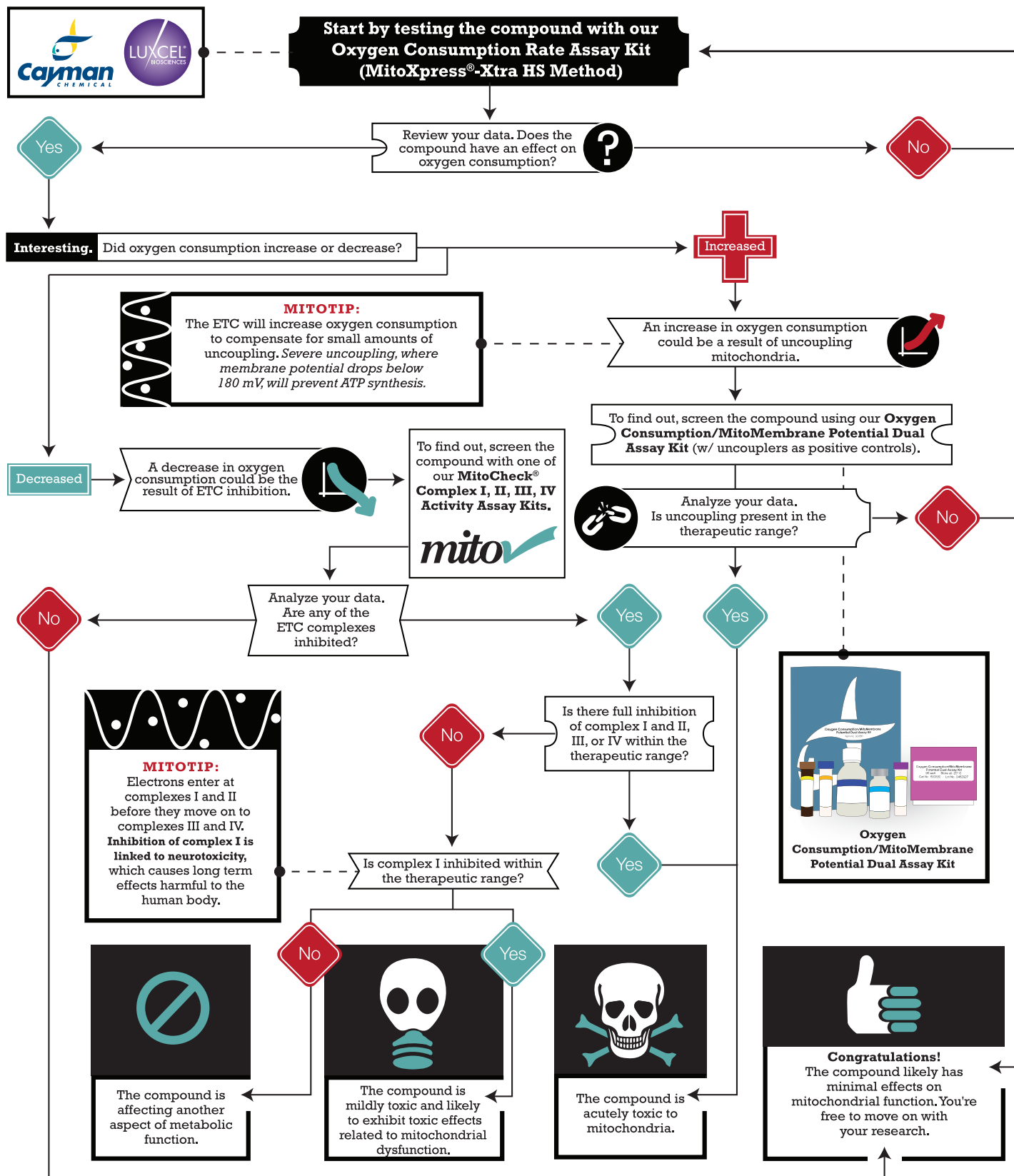
While mitochondria are critical in powering a number of cellular processes, they are also uniquely adapted to aid the cell in functions that are independent of ATP synthesis. The recent edition of Bioenergetics 4 is a comprehensive resource for describing these detailed and complex mechanisms.<sup>17</sup> Within this issue 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.

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# Evaluating Mitochondrial Toxicity

Since mitochondrial toxicity can affect normal heart, liver, and brain function, a potentially therapeutic compound must be screened for mitochondrial toxicity before being considered for clinical applications. The process below walks you through how to evaluate mitochondrial toxicity with Cayman's line of assay kits.

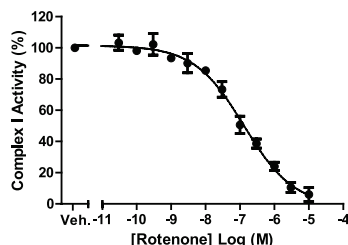


# MitoCheck® ETC Activity Assays

- Screen compounds for potential ETC inhibition
- Plate-based, colorimetric measurement
- Measure ETC activity in isolated mitochondria
- Isolated bovine heart mitochondria included in each kit

## MitoCheck® Complex I Activity Assay Kit

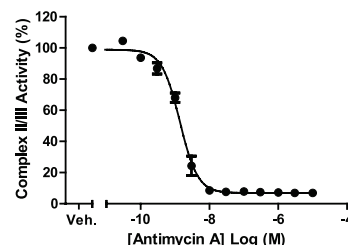
700930



- No need to pre-incubate with antibodies
- Suitable for high-throughput screening
- MitoCheck® Mitochondrial (Tissue) Isolation Kit (Item No. 701010) available to aid in mitochondrial tissue isolation

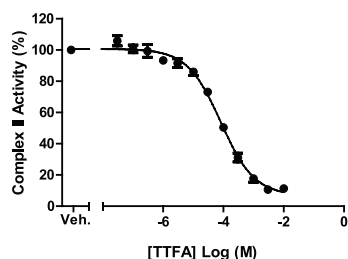
## MitoCheck® Complex II/III Activity Assay Kit

700950



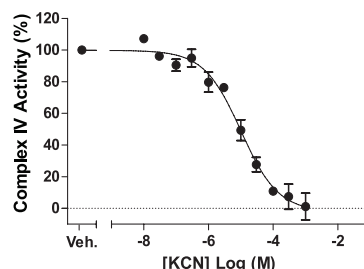
## MitoCheck® Complex II Activity Assay Kit

700940



## MitoCheck® Complex IV Activity Assay Kit

700990

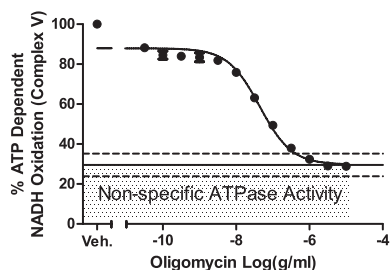


# MitoCheck® Complex V Activity

## MitoCheck® Complex V Activity Assay Kit

701000

- Measure complex V activity in isolated mitochondria
- Useful for screening compounds for complex V inhibition
- Suitable for high-throughput screening
- Plate-based, colorimetric measurement (340 nm)
- MitoCheck® Mitochondrial (Tissue) Isolation Kit (Item No. 701010) available to aid in mitochondrial tissue isolation

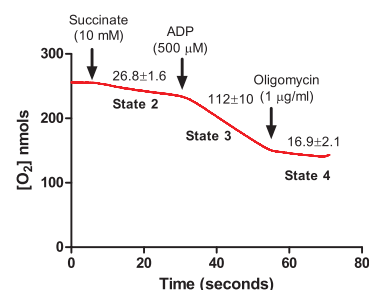


# MitoCheck® Mitochondrial Isolation

## MitoCheck® Mitochondrial (Tissue) Isolation Kit

701010

- Isolate coupled mitochondria from fresh tissue samples
- Isolated mitochondria can be used with MitoCheck® Complex I-V Activity Assays Kits and Citrate Synthase Activity Assay Kit (Item No. 701040)

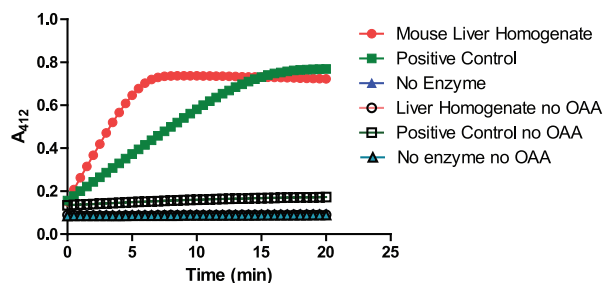




# MitoCheck® Citrate Synthase Activity

MitoCheck® Citrate Synthase Activity Assay Kit 701040

- Measure citrate synthase activity in isolated mitochondria, tissue and cell homogenate
- Assess mitochondrial content
- Suitable for high-throughput screening
- Plate-based, colorimetric measurement (412 nm)
- MitoCheck® Mitochondrial (Tissue) Isolation Kit (Item No. 701010) available to aid in mitochondrial tissue isolation

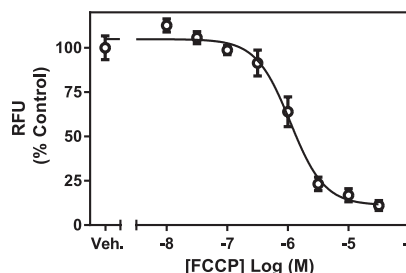


# Mitochondrial Membrane Potential

TMRE Mitochondrial Membrane Potential Assay Kit

701310

- Detect mitochondrial membrane potential as an indicator of mitochondrial health
- Utilize tetramethylrhodamine ethyl ester (TMRE), a fluorescent, lipophilic, cationic dye
- Staining in less than 1 hour



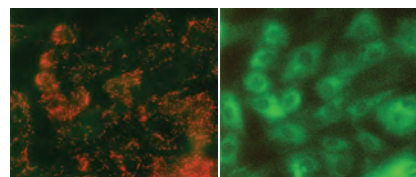
Mitochondrial Membrane Potential			
Item No.	Item Name	Key Information	Sizes
15003	JC-1	Cationic dye used to study mitochondrial integrity in the context of cellular apoptosis; changes fluorescence characteristics with alteration in mitochondrial membrane potential ( $\Delta\Psi_M$ )	1 mg • 5 mg 10 mg

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	Inhibits the mitochondrial $F_1F_0$ 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

JC-1 Mitochondrial Membrane Potential Assay Kit

10009172

- Measure mitochondrial membrane potential as an indicator of cell health
- Utilize JC-1, a fluorescent, lipophilic, cationic dye
- Red fluorescence indicates healthy mitochondria and green fluorescence indicates mitochondria in poor health
- Staining in less than 1 hour



H9C2 cells stained with JC-1 followed by FCCP treatment. Panel A: Cells prior to FCCP treatment shows JC-1 J-aggregates (red) accumulated in the mitochondria. Panel B: Cells subsequent to FCCP treatment shows diffuse JC-1 J-monomers (green) in the mitochondria.

## Mitochondrial Inhibitors

ETC Inhibitors			
Item No.	Item Name	Key Information	Sizes
11898	Atpenin A5	Selectively inhibits succinate dehydrogenase (complex II)	250 µg • 1 mg
15159	HQNO	Blocks mitochondrial complex III	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 complex I of the mitochondrial ETC	1 mg • 5 mg
13995	Rotenone	Irreversible inhibitor of complex I of the mitochondrial respiratory chain	1 g • 5 g • 10 g • 25 g
15517	2-Thenoyltrifluoroacetone	Irreversible inhibitor of complex II (succinate dehydrogenase) of the mitochondrial respiratory chain	10 g • 25 g • 50 g • 100 g

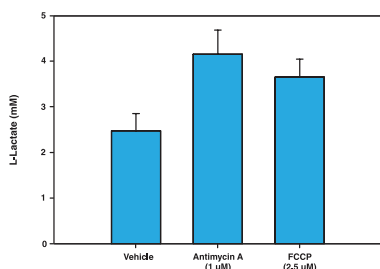
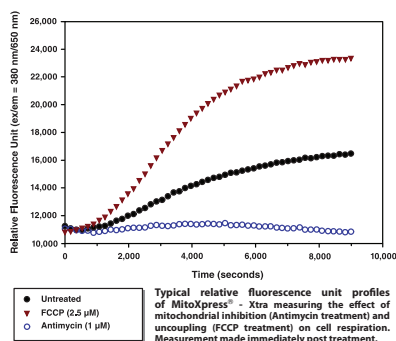
# Oxygen Consumption Rate Assays - in partnership with



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. 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 OCR in living cells in a 96-well plate format. The kits include a mitochondrial ETC inhibitor for use as a positive control and an oxygen depletion enzyme for use as a reference. Two of these novel kits, Item Nos. 601060 and 600880, combine the probe with additional readouts to offer a multiplex assessment of mitochondrial performance.

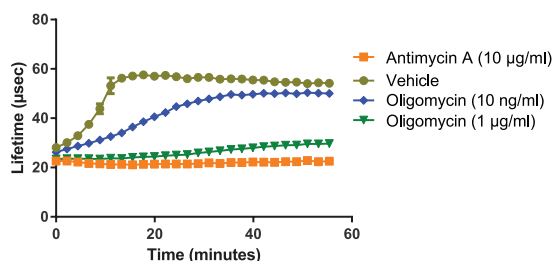
## Oxygen Consumption/Glycolysis Dual Assay Kit 601060

- Measure both oxygen consumption and glycolysis
- Utilize MitoXpress®-Xtra, a phosphorescent oxygen probe
- Includes antimycin A, an inhibitor of oxygen consumption, as a control



## MitoCheck® Mitochondrial OCR Assay Kit 701170

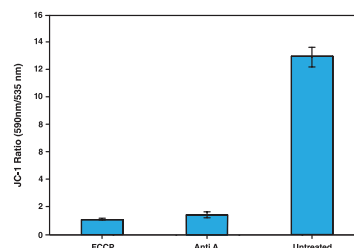
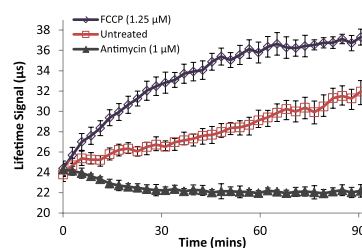
- Measure oxygen consumption rate (OCR) directly in freshly isolated mitochondria
- Useful for screening mitochondrial inhibitors and uncouplers
- Suitable for high-throughput screening
- Plate-based, fluorometric or time-resolved, fluorometric measurement (ex 380 nm, em 650 nm)
- MitoCheck® Mitochondrial (Tissue) Isolation Kit (Item No. 701010) available to aid in mitochondrial tissue isolation



**Sample Data.** Oxygen consumption is measured in isolated mouse liver mitochondria (0.12 mg/ml) in the presence the complex V inhibitor oligomycin (1 μg/ml, 10 ng/ml), the complex III inhibitor antimycin A (10 μM) and vehicle control. The difference in oxygen consumption rates in the absence and presence of oligomycin indicate that these mitochondria are coupled.

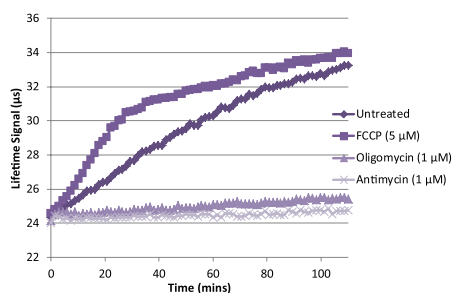
## Oxygen Consumption/MitoMembrane Potential Dual Assay Kit 600880

- Measure both oxygen consumption rate (OCR) and mitochondrial membrane potential
- Utilize MitoXpress®-Xtra, a phosphorescent oxygen probe
- Employ the cationic dye, JC-1, to determine mitochondrial membrane potential
- Includes antimycin A, an inhibitor of oxygen consumption, as a control
- Includes glucose oxidase as a reference for oxygen depletion



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

- Measure oxygen consumption rate (OCR) without the need for an oxygen electrode
- Utilize MitoXpress®-Xtra, a phosphorescent oxygen probe
- Includes antimycin A, an inhibitor of oxygen consumption, as a control
- Includes glucose oxidase as a reference for oxygen depletion



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	10 mg • 25 mg • 50 mg
9001873	L-Propionylcarnitine (chloride)	A carnitine derivative formed by carnitine acetyltransferase during $\beta$ -oxidation of uneven chain fatty acids that plays a role in mitochondrial metabolism	25 mg • 50 mg • 100 mg

Additional Inhibitors			
Item No.	Item Name	Key Information	Sizes
16605	Actinonin	An aminopeptidase inhibitor that targets the plastid peptide deformylase	5 mg • 25 mg
14804	Atractyloside (potassium salt)	Prevents mitochondrial ATP synthesis by inhibiting ADP/ATP translocases, which are responsible for the exchange of adenine di- and triphosphates across the inner mitochondrial membrane	500 $\mu$ g • 1 mg
17265	BI-6C9	Inhibits tBid-mediated apoptosis ( $K_d$ = 20 $\mu$ M), blocking the release of both cytochrome c and second mitochondrial-derived activator of caspase from mitochondria	1 mg • 5 mg • 10 mg
15611	CGP 37157	A selective inhibitor of the mitochondrial sodium-calcium exchanger ( $IC_{50}$ = 0.36 $\mu$ M in isolated mitochondria)	10 mg • 50 mg
16981	CPI-613	Inhibits $\alpha$ -ketoglutarate dehydrogenase; shown to induce mitochondrial ROS	5 mg • 10 mg • 25 mg 50 mg
11969	(+)-Etomoxir (sodium salt)	Irreversibly inhibits CPT1, a mitochondrial enzyme involved in $\beta$ -oxidation ( $IC_{50}$ = 5-20 nM in rat liver)	5 mg • 10 mg • 25 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 $\mu$ M) and mammalian cells ( $IC_{50}$ = ~50 $\mu$ M); prevents apoptosis by inhibiting mitochondrial outer membrane permeabilization	5 mg • 10 mg • 25 mg 50 mg
15997	Mildronate (hydrate)	An inhibitor of carnitine biosynthesis	10 mg • 25 mg • 50 mg 100 mg
16982	Perhexiline (maleate)	Inhibits CPT1 and CPT2 ( $IC_{50}$ s = 77 and 79 $\mu$ M in rat heart, respectively)	1 mg • 5 mg • 10 mg
15550	UCF 101	Selectively inhibits the proteolytic activity of Omi/HtrA2 ( $IC_{50}$ = 9.5 $\mu$ M)	1 mg • 5 mg • 10 mg 25 mg
16980	UK 5099	Potently inhibits the mitochondrial pyruvate carriers, decreasing pyruvate-dependent oxygen consumption by rat heart mitochondria ( $IC_{50}$ = 50 nM)	1 mg • 5 mg • 10 mg 25 mg



QUESTIONS FROM THE FIELD

## How do the ETC 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.

# Glycolysis

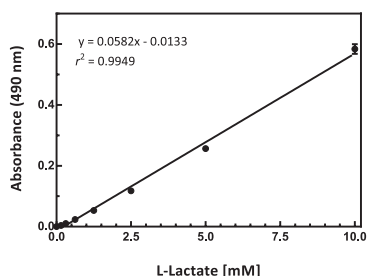
## Glycolysis Cell-Based Assay Kit

600450

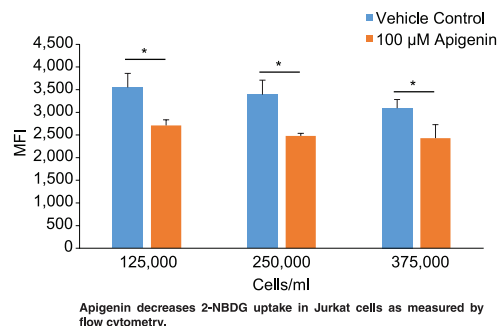
## Glucose Uptake Cell-Based Assay Kit

600470

- Detect L-lactate, the end product of glycolysis
- Can be adapted to high-throughput screening
- Measure L-lactate in cell culture supernatant down to 156  $\mu$ M



- Measure glucose uptake by cultured cells
- Employ 2-NBDG, a fluorescent deoxyglucose analog
- An inhibitor of glucose transport is included as a control
- Convenient tool for studying modulators of glucose uptake



## Isolated Mitochondria vs. Mitochondria in Cells

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

	Isolated Mitochondria	Whole Cells	Rationale
Correlate OCRs with ETC activity	✓✓	✓	OCR correlates with ETC activity in both isolated and cellular mitochondria, but there may be other sources of oxygen consumption in whole cells; this requires additional experiments to control for background OCRs
Can measure respiratory control ratio (RCR) (i.e., integrity of the inner mitochondrial membrane, or "health" of the mitochondria)	✓✓	✓	RCR can be measured in fresh isolated mitochondria or whole cells, but the measurement in whole cells is not as accurate since oligomycin (a complex V inhibitor) needs to be present
Assay individual enzymes of the ETC	✓✓	—	Assaying individual ETC enzymes is not possible in whole cells
Measure membrane potential	✓✓	✓	Membrane potential can be measured in both isolated and whole cells, but there is a chance of cellular membrane potential interference in whole cells
Measure glycolytic function (lactate production)	—	✓✓	Measuring glycolytic function is not possible in isolated mitochondria because glycolysis occurs in the cytoplasm



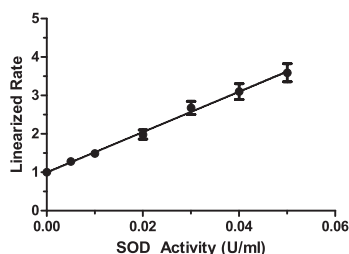
# Oxidative Stress

SOD Antibodies			
Item No.	Item Name	Applications	Species Reactivity
10011388	Cu/Zn SOD (human) Polyclonal Antibody	ELISA, IHC, IP, WB	(+) human, bovine, canine, coral, hamster, monkey, mouse, ovine, porcine, rabbit, rat
10011390	Mn SOD (human) Polyclonal Antibody	IHC, IP, WB	(+) human, bovine, canine, chicken, gerbil, guinea pig, hamster, monkey, mouse, ovine, porcine, rabbit, rat, <i>Xenopus</i>
10011389	Mn SOD (rat) Polyclonal Antibody	ELISA, IHC, IP, WB	(+) human, bovine, canine, chicken, <i>Drosophila</i> , guinea pig, hamster, monkey, mouse, ovine, porcine, rabbit, rat, <i>Xenopus</i>

## Superoxide Dismutase Assay Kit

706002

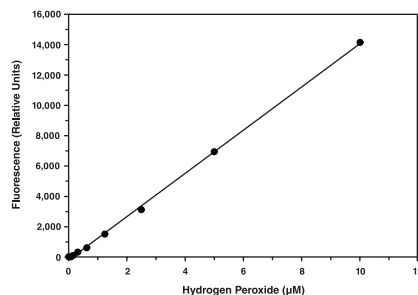
- Measure copper/zinc, manganese, and iron superoxide dismutase (SOD) in tissues, plasma, serum, and cell lysates
- Assay 41 samples in duplicate
- Measure SOD activity down to 0.005 U/ml



## Hydrogen Peroxide Cell-Based Assay Kit

600050

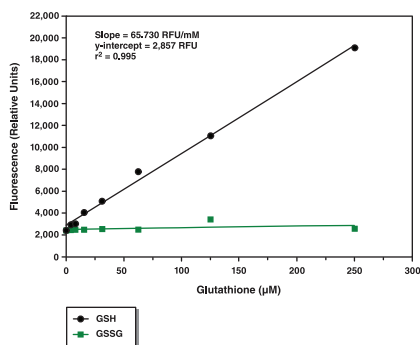
- A simple fluorometric assay for the quantification of extracellular  $H_2O_2$
- Utilize ADHP, a sensitive and stable probe for  $H_2O_2$
- Catalase, an  $H_2O_2$  scavenger, is included as a control for specificity
- Rapid assay; results in under 1 hour



## Glutathione Cell-Based Detection Kit (Blue Fluorescence)

600360

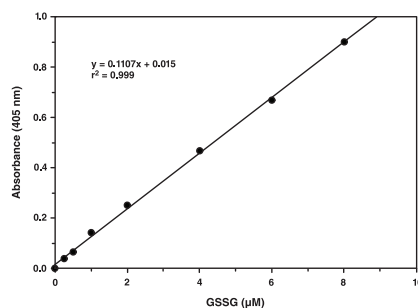
- Utilize monochlorobimane (MCB), a highly fluorescent GSH probe
- Includes a standard curve for accurate quantification of GSH from cell lysates
- Rapid assay; get results in 2 hours



## Glutathione Assay Kit

703002

- Measure total, oxidized (GSSG), and/or reduced (GSH) glutathione in cell lysates, tissue homogenates, plasma and erythrocyte lysates, and serum
- Assay 40 samples in duplicate
- **Assay Range:** 0.25-8 µM (GSSG) or 0.5-16 µM (GSH)



# Researcher Spotlight

## 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 I 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 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.



## Renata Goncalves, Ph.D.

Postdoctoral Research Scholar

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

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

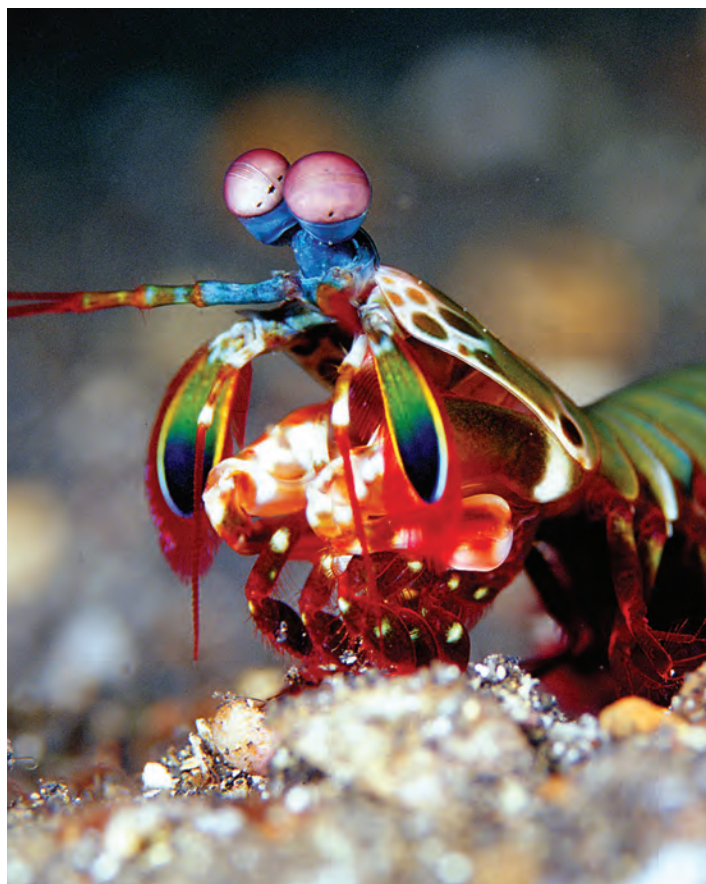
Want to have your research  
featured in the Cayman Currents?  
Send a brief background to  
[marketing@caymanchem.com](mailto:marketing@caymanchem.com)

## 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 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.

### THREE 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.



QUESTIONS FROM THE FIELD

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

While the bulk of the cellular OCR is mitochondrial, there are numerous intracellular reactions that require oxygen as a substrate. Therefore, when measuring mitochondrial OCR it is imperative that the background (non-mitochondrial) OCR is subtracted. The background OCR can be easily measured by fully inhibiting the mitochondrial OCR with an inhibitor such as antimycin A or potassium cyanide. Once established, the actual mitochondrial OCR provides a suitable starting point for determining mitochondrial toxicity. To provide more detailed information, researchers can measure the activity of each individual complex of the ETC using Cayman's MitoCheck® ETC Activity Assay Kits.



QUESTIONS FROM THE FIELD

### Why study toxicity in the mitochondria?

Mitochondrial toxicity 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. Additionally, 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.



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