Bioanalytical Redox Biology Core

UAB RESEARCH
The University of Alabama at Birmingham

UAB SCHOOL OF HEALTH PROFESSIONS
The University of Alabama at Birmingham

DEPARTMENT OF NUTRITION SCIENCES

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B.A.R.B. Bio-Analytical Redox Biology Core

The BARB Core facilitates the study of mitochondrial dysfunction and oxidative stress, which are key molecular processes contributing to both metabolic and vascular pathologies. The Core supports research activities around these common themes by providing technical services and support at three levels:

► Short-term or preliminary studies can be performed by the Core team, who are experts in these areas.

► The lab of the PI can perform part of the work, such as initial cell/tissue preparation, and then the Core will complete the study. In many cases, DRC investigators spend some time in the Core facility, being instructed on these procedures.

► The Core personnel, Doug Moellering PhD, will train designated individuals from DRC labs to become independent in the procedure of choice if that procedure will be established in the long-term goals of a given lab.

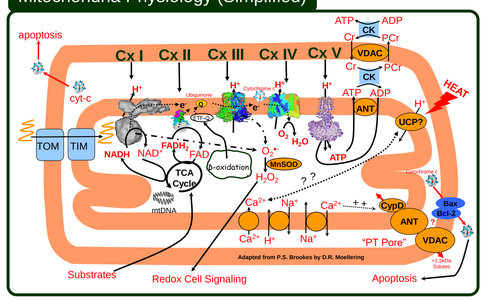
Services:

Mitochondrial Bioanalytical Services

Oxidative Stress (Redox) Measures

Bioenergetics: Cell Culture, permeabilized human skeletal muscle, tissue slices (amorphous in development)

Mitochondria Physiology (Simplified)



Mitochondrial Bioanalytical Services:

mtDNA Damage Analysis
Quantitative PCR Assay for DNA Damage

High Resolution Respirometry
Measure oxygen kinetics in well coupled highly functional isolated mitochondria from various species and tissues

Bioenergetics' Analysis of Tissues, Mitochondria, and Cells
Using a Seahorse XF24-3 instrument, we are able to measure oxygen consumption (as an indicator of oxidative phosphorylation simultaneously with extracellular acidification (as an indicator of glycolysis) in living cells and isolated mitochondria.

Mitochondrial Oxidative Phosphorylation Complex and other Mitochondrial Proteins' Activity Assays
As a complementary approach to measuring altered total oxygen consumption using the Oxygraph-2k respirometer or the XF24 analyzer, in either pathological or therapeutic samples compared to controls, individual OxPhos complexes can be measured using a spectrophotometer to ascertain possible specific mechanisms and altered individual complex activities contributing to those differences.

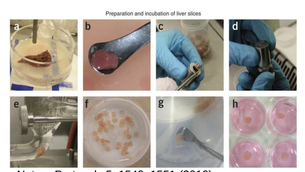
Mitochondrial Complex I (NADH-Ubiquinone oxidoreductase) Assay
The principle of this assay is based on the consumption of NADH

Mitochondrial Complex IV (Cytochrome c oxidase) Assay
The principle of this assay is based on the oxidation of reduced cytochrome c that is followed at a spectrophotometric absorbance of 550nm.

Mitochondrial Citrate Synthase Assay
routinely measured as an index for mitochondrial content

Mitochondria Isolation and Preparation
Standardized, reproducible isolation of highly functional and well coupled mitochondria from different species and tissues

Bioenergetic Analyses of Tissue Slices (in development)



Nature Protocols 5, 1540–1551 (2010)

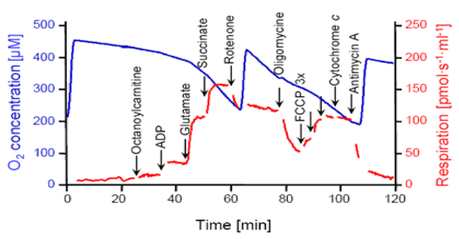

Krumdieck Tissue Slicer

Precision-cut tissue slices (PCTS), with reproducible, standardized, and defined thickness allow for *ex vivo metabolic organ* analyses of tissues/cells while maintaining their native intercellular, multicellular , and cell-matrix architecture.

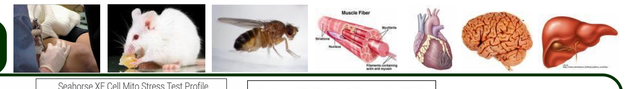
Advantages: Slices may be made from any fresh tissue & serial slices may be used for other complementary assays. Liver slices are currently being prepared using a coring tool and a specially designed Krumdieck Tissue Slicer. Amorphous tissues may be embedded in cylinders of agarose before slicing.

High Resolution Respirometry


Measure oxygen kinetics in reproducibly isolated mitochondria from various species and tissues using an Oroboros Oxygraph-2K



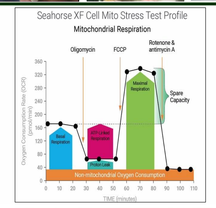
Bioenergetics' Analyses of Cells and Isolated Mitochondria

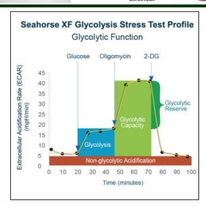


Using a Seahorse XF24 instrument, we are able to measure energy utilization in living cells and in isolated mitochondria from a variety of species and tissue sources, simultaneously quantifying aerobic respiration from mitochondria and glycolysis in real time. The technology offers a robust and simple method for studying substrate utilization, mitochondrial function, energy expenditure and cell quality in microplates, without the use of large number of cells, mitochondria, flasks, electrodes, dyes, radioactive materials or lysis of cells that is typical of other methods.

SeahorseBioscience

The XF24 Analyzer measures oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) at intervals of approximately 2-5 minutes. OCR is an indicator of mitochondrial respiration, and ECAR is predominately the result of glycolysis.

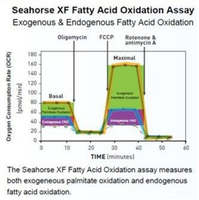




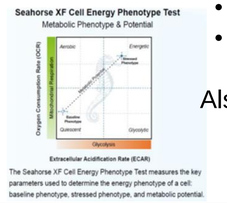
Seahorse XF Consumables:

- Media
- Kits
- Inhibitors

Also Available

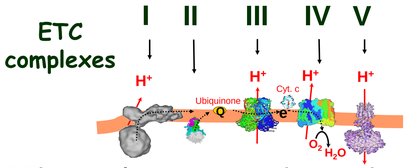


The Seahorse XF Fatty Acid Oxidation assay measures both exogeneous palmitate oxidation and endogenous fatty acid oxidation.



The Seahorse XF Cell Energy Phenotype Test measures the key parameters used to determine the energy phenotype of a cell: baseline phenotype, stressed phenotype, and metabolic potential.

OxPhos and other protein activity assays



OxPhos complexes are measured spectrophotometrically to ascertain possible specific mechanisms and altered individual complex activities contributing to those differences.

Advantages: can be measured on snap frozen samples while respirometry must be completed using only freshly isolated samples.

Citrate Synthase is routinely measured as an index for mitochondrial content.

Oxidative & Stress (Redox) Measures:

Elevated reactive oxygen/nitrogen species (RO[N]S) levels can lead to oxidative damage of DNA/RNA, proteins and lipids which may lead to many possible outcomes including apoptosis, dysfunction, or altered bioenergetics and cellular signaling. Cells have developed several mechanisms to counteract elevated RO[N]S levels such as a thiol reducing buffer composed of cellular thiol levels (glutathione and thioredoxin) for the maintenance of the reduction-oxidation (Redox) state of the cell, and enzymes to remove ROS (catalase, superoxide dismutase and glutathione peroxidase).

Glutathione (Total oxidized [GSSG] & reduced [GSH])
Using a "Tietze" recycling spectrophotometric assay, we are able to measure oxidized and reduced levels of glutathione.

RedOx Enzymes' Kinetic Activity
Enzyme-specific spectrophotometric kinetic activity measures (please inquire)

mtDNA content and Damage
Quantitative PCR Assay for mitochondrial DNA (mtDNA) content and damage. Undamaged DNA yields a full length product while 'damaged' mtDNA causes the polymerase to stall or fall off, resulting in decreased amounts of full length amplification products indicating higher levels of mtDNA damage.

High throughput microplate assays: Absorbance, Fluorescence, Luminescence

Applications: using cells and isolated mitochondria to measure ROS production, mitochondrial membrane potential, and more.

Take3 Multi-Volume Plate: sixteen 2 µL micro spots for low volume DNA/RNA 260 nm measurements. Saves precious samples, very fast and simple process

Assays in Development

- **Reactive Oxygen Species (ROS) kinetics**
As a paired approach to measuring mitochondrial respiration using isolated mitochondria and cells, we will develop assays to measure ROS production under various conditions and bioenergetic states
- **Mitochondrial Membrane Potential (ΔΨ)**
As a paired approach to measuring mitochondrial respiration using isolated mitochondria and cells, we will develop assays to measure mitochondrial membrane potential (delta psi).
- **Lipid Hydroperoxide & 8-Isoprostane Assay**
Lipid peroxidation quantification as a direct measure of oxidative injury in tissues, cultured cells, or biological fluids (plasma). More accurate than measuring malondialdehyde (MDA) or 4-HNE alone.
- **Rapid DNA, RNA and Protein Quantification**
- **ATP and Phosphocreatine quantification**
- **Cortisol Assay – saliva, serum, hair, finger/toe nails**
- **Nitrate/Nitrite Assay; Viability Assays**

BARB Core Acknowledgments

UAB DIABETES RESEARCH CENTER P30DK079626
The University of Alabama at Birmingham

UAB COMPREHENSIVE DIABETES CENTER
The University of Alabama at Birmingham

UAB NUTRITION OBESITY RESEARCH CENTER

UAB CENTER for FREE RADICAL BIOLOGY

UAB COMPREHENSIVE NEUROSCIENCE CENTER
The University of Alabama at Birmingham

UAB CENTER FOR EXERCISE MEDICINE
The University of Alabama at Birmingham

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