

# Agilent Technologies: Seahorse XF Introduction

Tools for investigating cell metabolism



Metabolism is measured via the *rates of change* in **pH** and **Oxygen concentration** 



### Measuring functional metabolism with the Seahorse XF Analyzer



**Oxygen Consumption Rate (OCR):** Measurement of mitochondrial respiration

Extracellular Acidification Rate (ECAR): Indicator of glycolysis

For Research Use Only. Not for use in diagnostic procedures.



Agilent Technologies

# Measuring Mitochondrial Respiration and Glycolysis in a Microplate





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## Automated compound injections enable kinetic, functional data



- •4 compound injection ports per well
- •Add inhibitors, stimulants, substrates, etc.
- Injections are defined by the user









# Measuring mitochondrial respiration and glycolysis in a microplate with live cells

MIX	<ul> <li>Gentle up/down</li> <li>3 minutes standard</li> <li>Restores O2 and pH levels</li> </ul>	
WAIT	<ul> <li>Probes remain Up</li> <li>Only in 24-well protocols</li> <li>Cycle ~ 3 tire</li> </ul>	nes
MEASURE	<ul> <li>Probes down- microchamber formed</li> <li>2-3 minutes standard</li> </ul>	
INJECT	<ul> <li>Manifold system injects all ports of a given position "A" or "B" etc.</li> <li>then Cycle</li> </ul>	



### Sequence of Operations and Raw Data: pH vs. time → ECAR





### Automatic Calculation of Extracellular Acidification Rate (ECAR)





### Sequence of Operations and Raw Data: $O_2$ vs. time $\rightarrow$ OCR





### Automatic Calculation of Oxygen Consumption Rate (OCR)





### Seahorse XF Analyzers and Standardized Seahorse XF Assays





## The Mitochondrial Engine: Electron Transport Chain





### Agilent Seahorse XF Cell Mito Stress Test





Mitochondria have an energy reserve to handle acute stress demands

# Measure the key parameters of mitochondrial function:

- Basal Respiration
- ATP Production
- Maximal Respiration and Spare Capacity
- Proton Leak





# Agilent Seahorse XF Cell Mito Stress Test for Mitochondrial Function





Loft A et al., (2015) Genes. Dev.

Seahorse XF Cell Mito Stress Test reveals a link between rosiglitazone stimulation and metabolic profile in hMADs-derived brite adipocytes.



# Agilent Seahorse XF Cell Mito Stress Test for Mitochondrial Function





XF Cell Mito Stress Test reveals the dose-dependent susceptibility of breast cancer cells to polyunsaturated fatty acids as shown by a depression in all parameters of mitochondrial respiration.



# Agilent Seahorse XF Cell Mito Stress Test for Mitochondrial Function





Human liver-derived cells Kenna JG *et al.*, (2015) J Pharmacol Exp Ther.

Seahorse XF Cell Mito Stress Test reveals a dosedependent reduction in mitochondrial respiration in human liver-derived cells (Huh-7) following treatment with sitaxentan, an endothelin receptor antagonist.



#### CARDIOVASCULAR

#### Stress Test Sensitivity Reveals Sub-Lethal Stress Response and Mechanism



#### IMMUNO-METABOLISM Tailoring T Cells to Fight Cancer



**Distinct Signaling of Coreceptors Regulates Specific Metabolism Pathways and Impacts Memory Development in CAR T Cells** Kawaleka et al. *Immunity* 2016 February 16



# Real time metabolic analysis delivers a rapid and robust indicator of activation in minutes







Use injection ports to investigate modulators of T cell activation kinetics



www.agilent.com/chem/immunology



# A new approach to quantifying macrophage activation





## Agilent Seahorse XF Glycolysis Stress Test



# Measure the key parameters of glycolytic function:

- Non-glycolytic Acidification
- Glycolysis
- Glycolytic Capacity
- Glycolytic Reserve





Cells have an energy reserve to handle acute stress demands



#### CANCER XF Glycolysis Stress Test Measures Glycolytic Phenotype Susceptible to Buffer Therapy



Free Base Lysine Increases Survival and Reduces Metastasis in Prostate Cancer Model

Ibrahim-Hashim, et al., J Cancer Sci Ther 2011, S1



#### Agilent Seahorse XF Glycolytic Rate Assay





## Glycolytic Rate Assay : Determining the Rate



- Calculate mitoPER (using CCF and OCR)
- Subtract mitoPER from PER to obtain glycoPER





#### A431 cells: Highly glycolytic





#### Bovine Aortic Endothelial cells (BAEC) : Highly oxidative





### XF Glycolytic Rate Assay Report Generator outputs

		MCF7 Ctrl	MCF7Dox
Parameter	Units	Average	Average
Basal Glycolysis	pmol/min	140	294
Basal Proton Efflux Rate	pmol/min	236	419
% PER from glycolysis	%	59%	70%
Compensatory Glycolysis	pmol/min	379	838
mitoOCR/glycoPER	-	1.13	0.71
Post 2-DG acidification	pmol/min	81	99



#### Agilent Seahorse XF Real-Time ATP Rate Assay

An assay that quantifies ATP production rates from mitochondrial respiration and glycolysis simultaneously, in real time, and in live cells.

- 1. Is a powerful new measure of cell function.
- 2. Offers a new perspective on ATP dynamics and provides new insights into your research model.
- 3. Quantifies metabolic switching with a definitive view of cell energy metabolism and identifies pathway liabilities.





# Estrogen Receptor Positive (ER+) Breast Cancer Cell Lines are More Oxidative than ER- or Control Cell Lines



#### Energetic map





#### Agilent Seahorse XF Substrate Oxidation Stress Test









#### IMMUNOLOGY Spare Capacity Affects Memory T Cell Development and Survival





Mouse Lymphocytes



*Mitochondrial respiratory capacity is a critical regulator of CD8+ T cell memory development* van der Windt, JW, ... and Pearce, EL, Washington School of Medicine. *Immunity* 2012 Jan 27



IMMUNOLOGY Spare Capacity in CD8+ T Cells is Dependent on Mitochondrial Fatty Acid Oxidation





*Mitochondrial respiratory capacity is a critical regulator of CD8+ T cell memory development* van der Windt, JW, ... and Pearce, EL, Washington School of Medicine, *Immunity* 2012 Jan 27



#### Agilent Seahorse XF Palmitate Oxidation Stress Test







