Agilent
Trusted Answers

Agilent Seahorse XF细胞能量代谢分析技术及应用

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技术支持
广州皇河仪器科技有限公司

HuangHe
皇河科技 Make Research Easy

使命：让科研更简单

目标：成为最专业的科学实验解决方案服务商

1 通用实验仪器

天平、pH计、移液器、纯水/超纯水系统、培养箱、灭菌器、低温冷藏设备等；.

2 理化实验仪器

滴定仪、水分仪、微波合成仪、制备色谱系统、快速蒸发系统、氮吹仪、旋转蒸发仪、冻干机、真空泵等；

3 生命科学实验仪器

流式细胞仪、实时无标记细胞分析系统、活细胞成像系统、酶标仪、洗板机、荧光定量PCR系统、seahorse、细胞计数仪等；

4 试剂耗材

生物实验耗材试剂，流式抗体试剂，微球，高品质吸头。

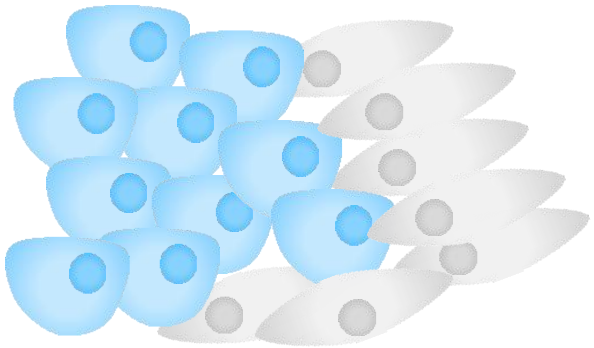
Contents

检测原理介绍

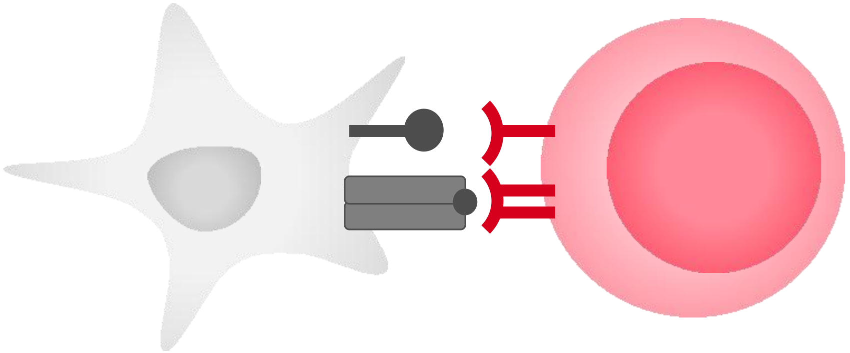
Agilent Seahorse XF技术

揭示驱动细胞功能变化的机制

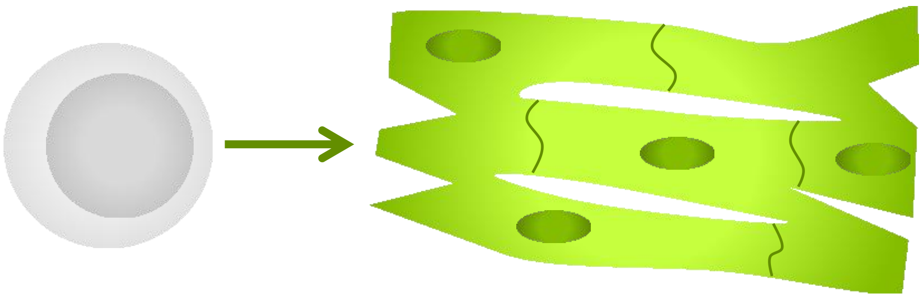
肿瘤细胞增殖



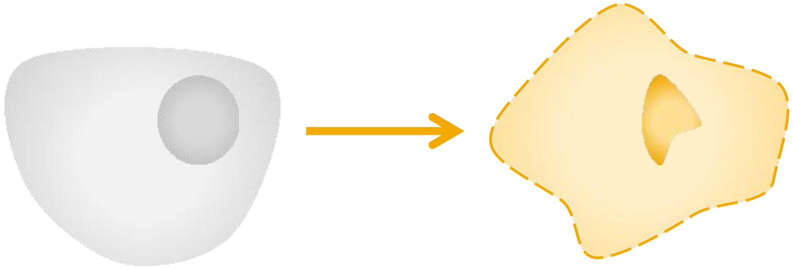
免疫细胞激活



干细胞分化



细胞死亡



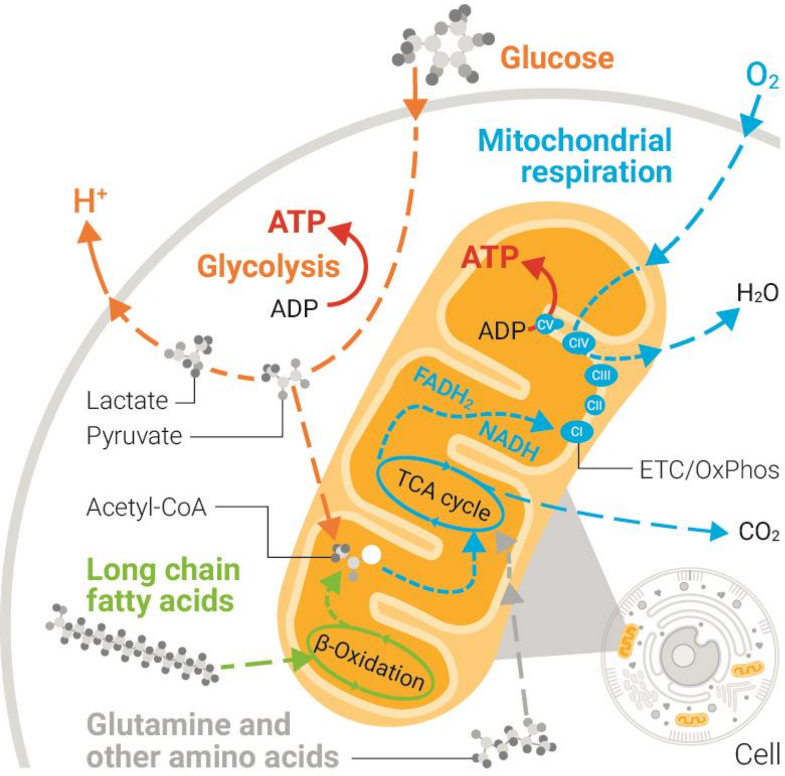
Agilent Seahorse XF技术测量细胞内两条主要的能量产生途径

糖酵解

Anaerobic conversion of glucose to lactate

ECAR
(mpH/min)
Extra Cellular Acidification Rate
细胞外酸化速率

PER
(pmol [H^+]/min)
Proton Efflux Rate
质子流出速率



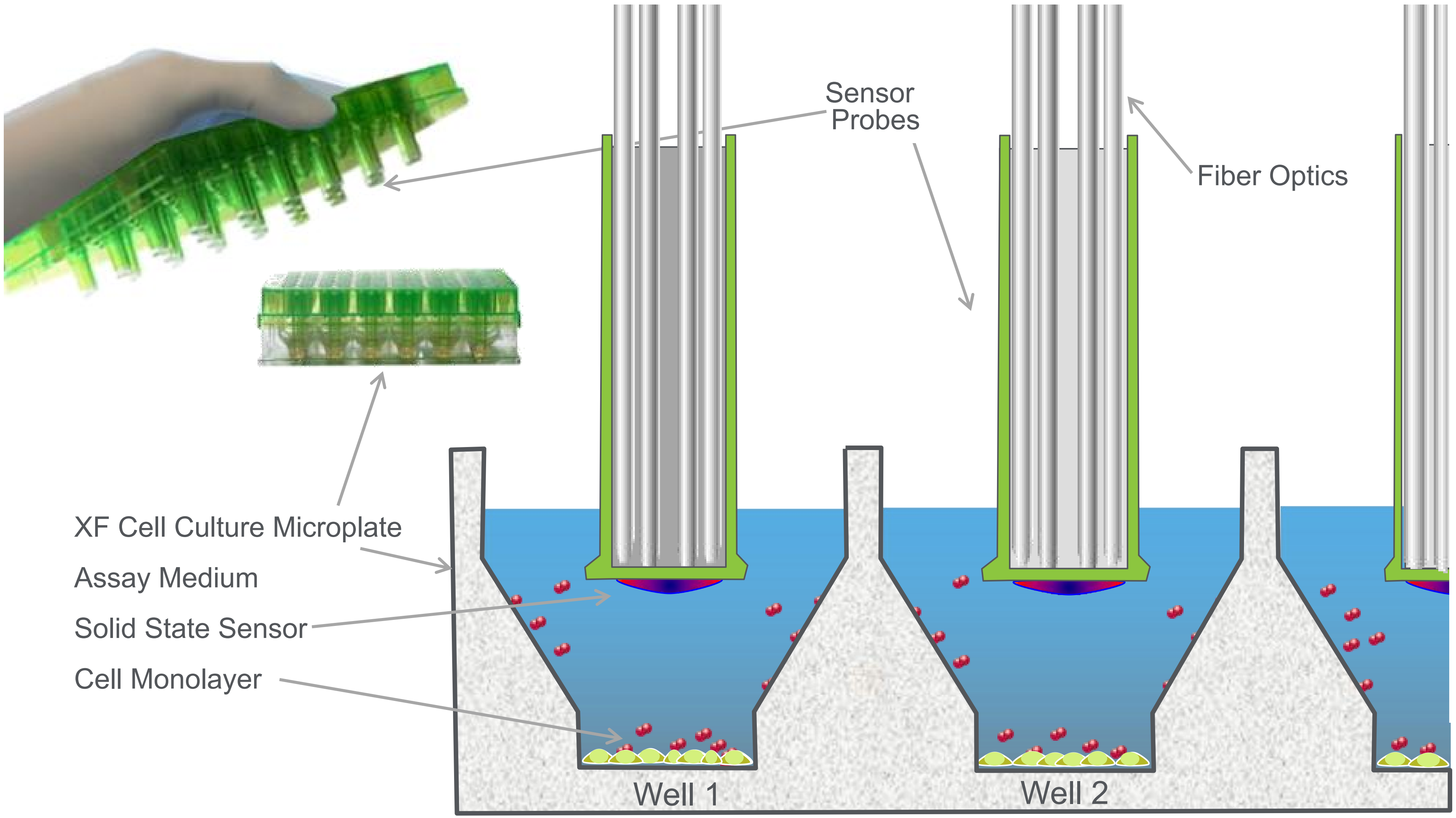
线粒体呼吸

Oxidation of glucose, fatty acids, amino acids

OCR
pmol [O_2]/min

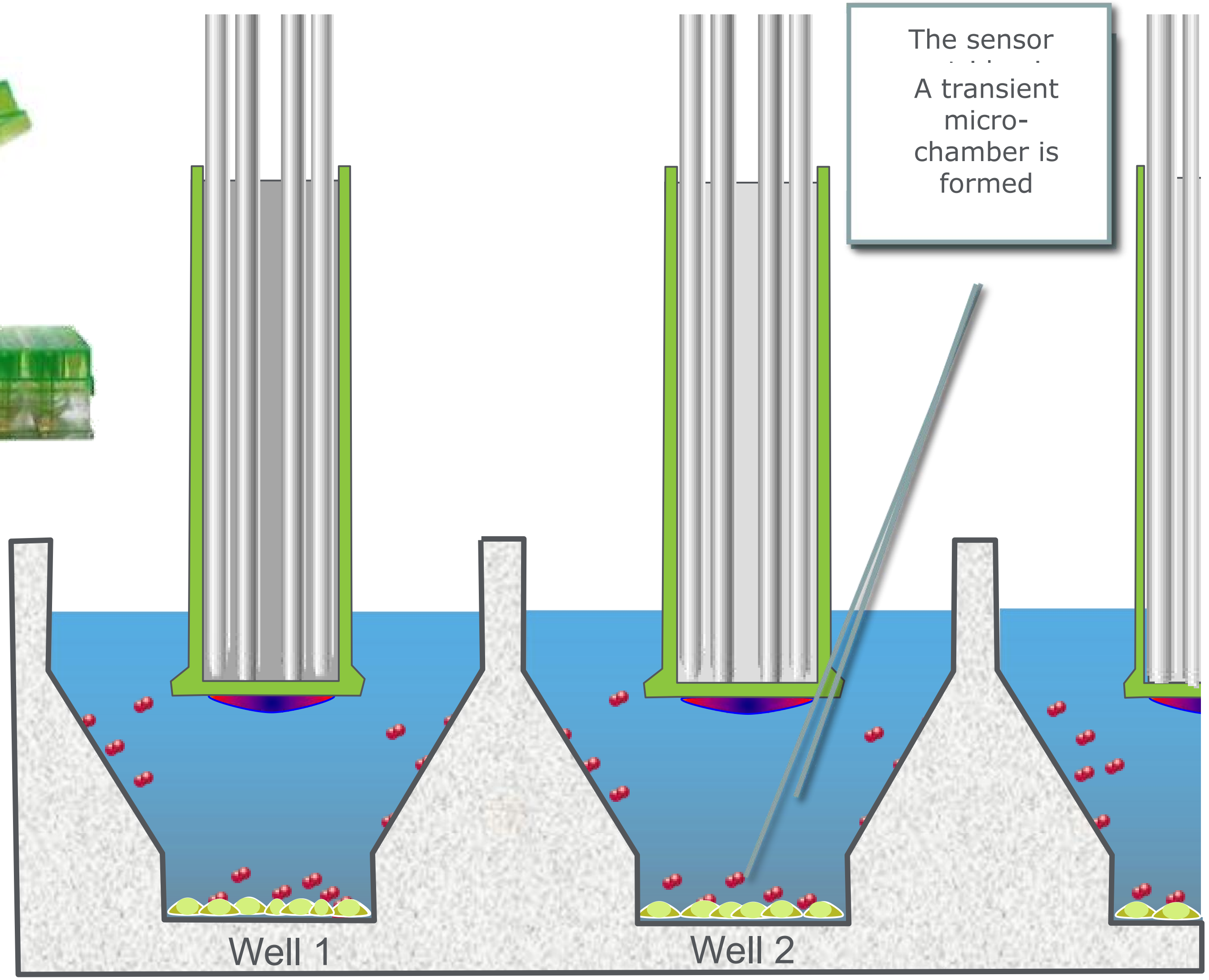
Oxygen Consumption Rate
氧消耗速率

在微孔板上测量线粒体呼吸和糖酵解

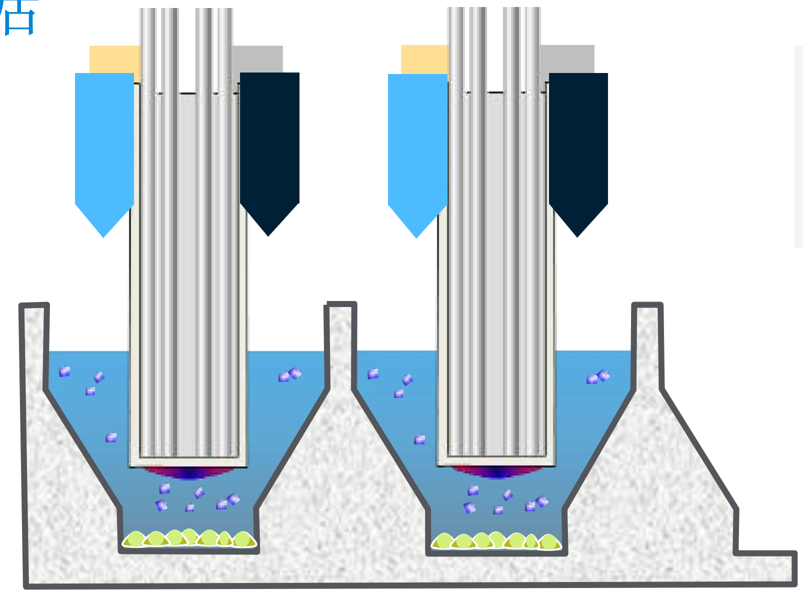


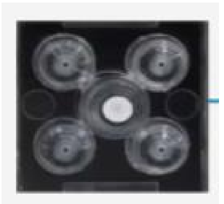
在微孔板上测量线粒体呼吸和糖酵解

The transient micro-chamber enables rapid measurement of oxygen consumption and extracellular acidification in minutes

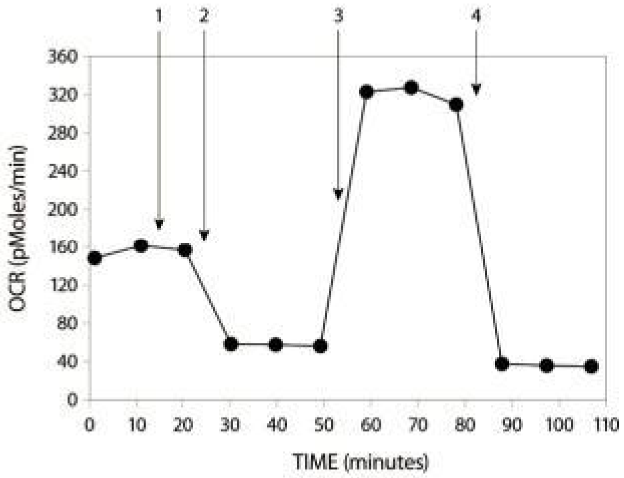


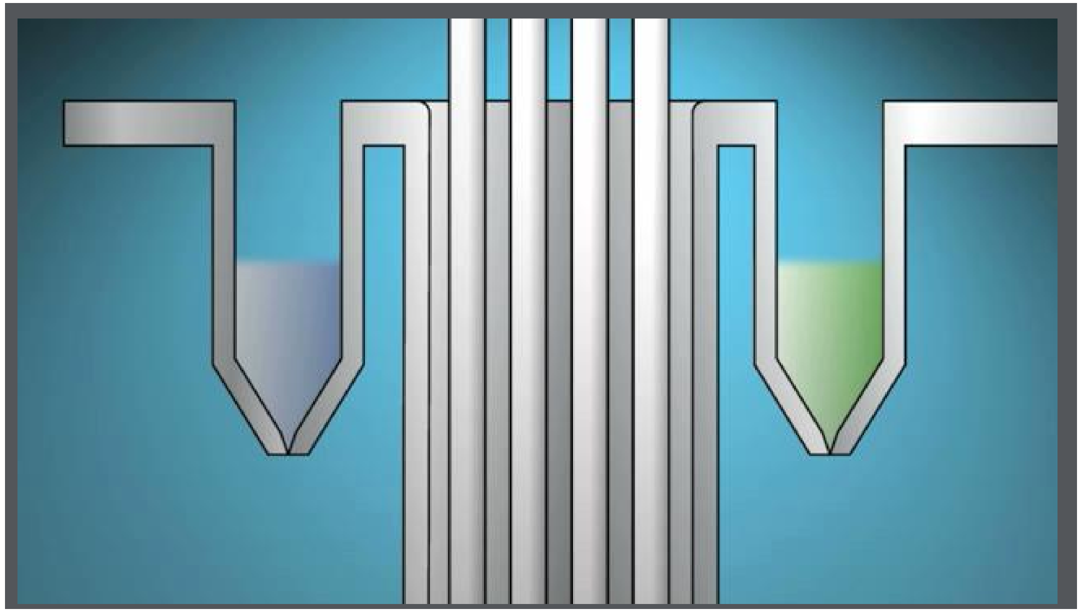
实时自动加药，获取动态功能性数据



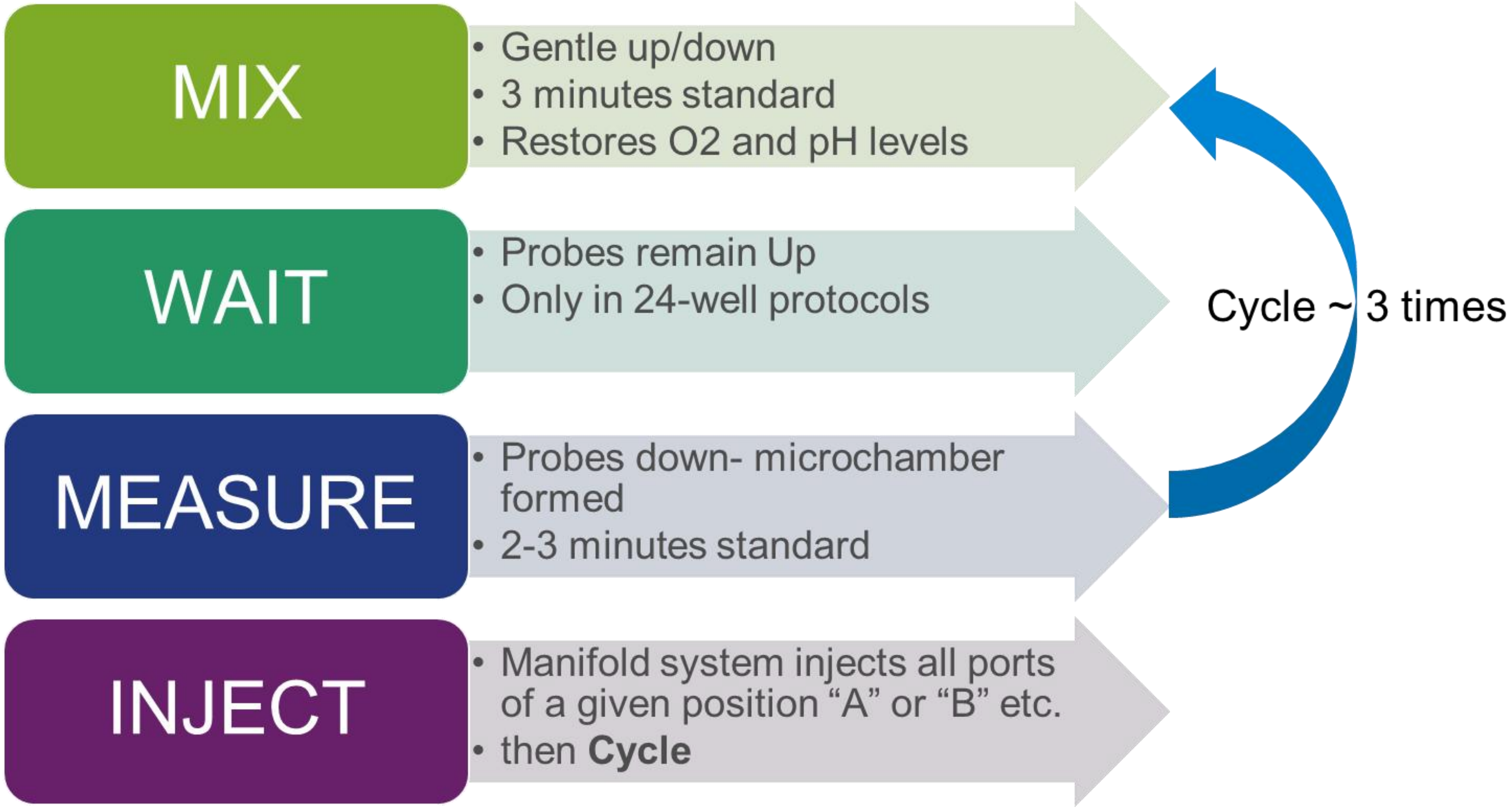


- 每个细胞孔对应4个加药孔
- 添加抑制剂、刺激剂、底物等
- 用户自定义加药

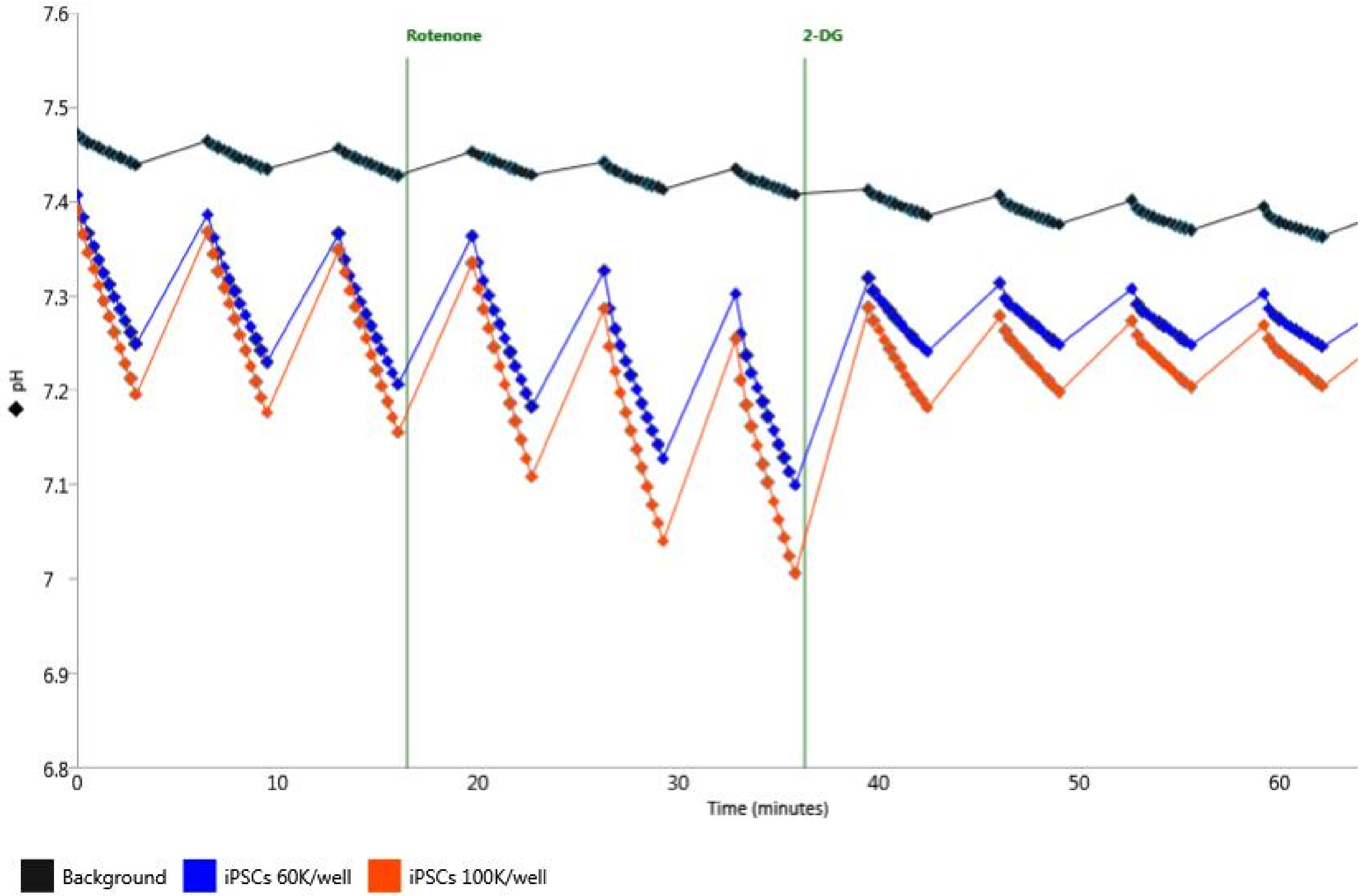




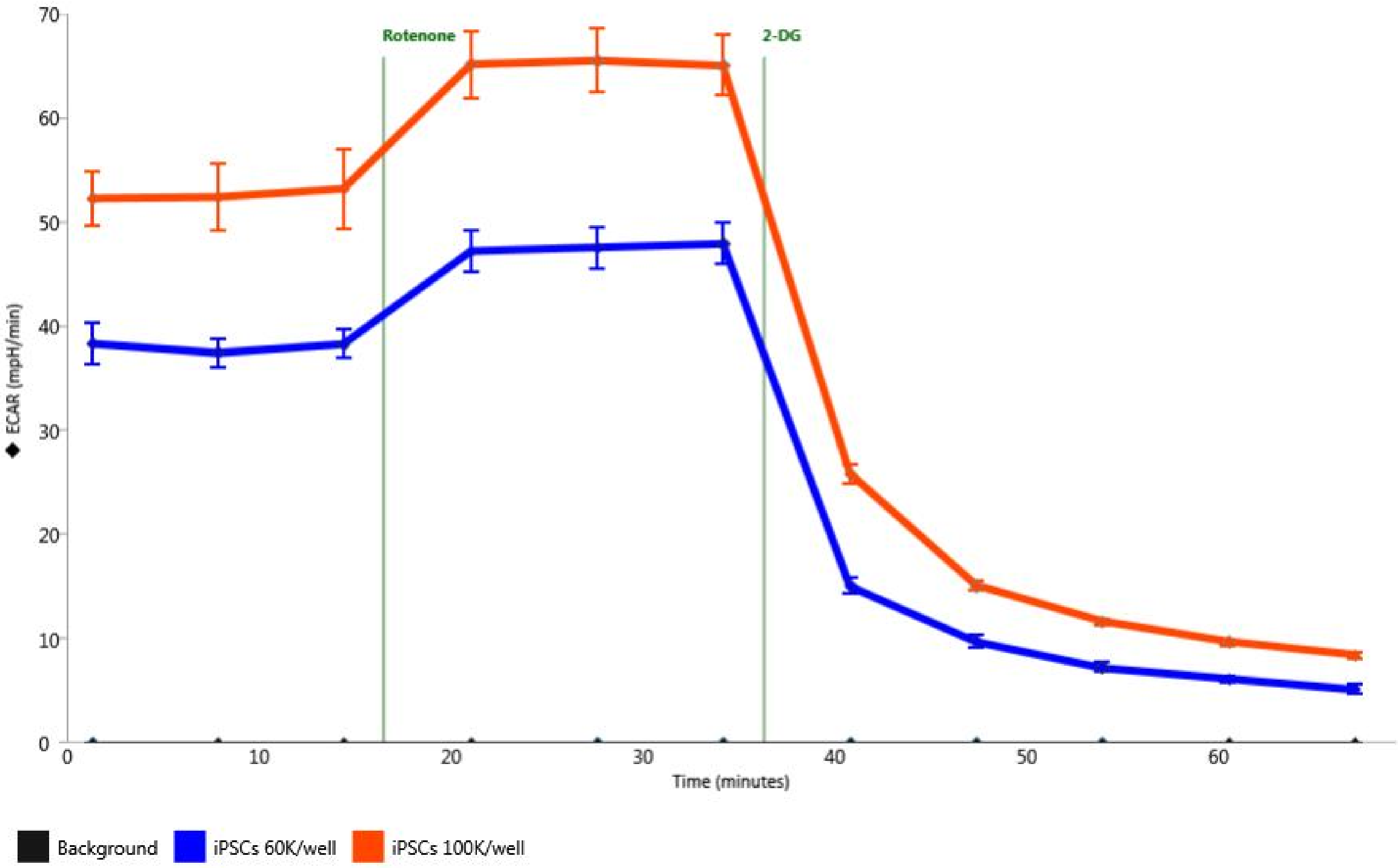
在微孔板上测量活细胞线粒体呼吸和糖酵解



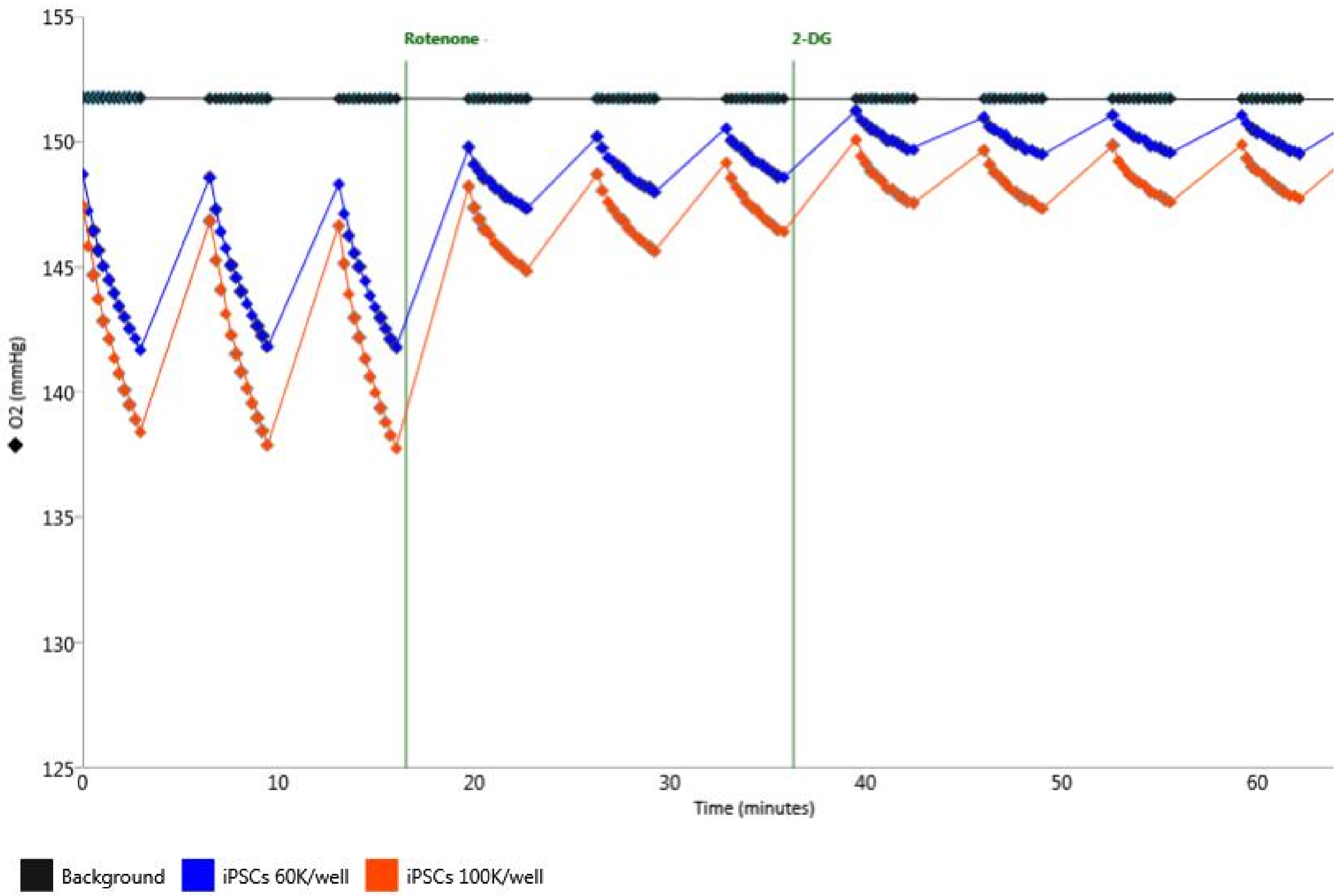
Sequence of Operations and Raw Data: pH vs. time ➜ ECAR



Automatic Calculation of Extracellular Acidification Rate (ECAR)



Sequence of Operations and Raw Data: O_2 vs. time ➜ OCR



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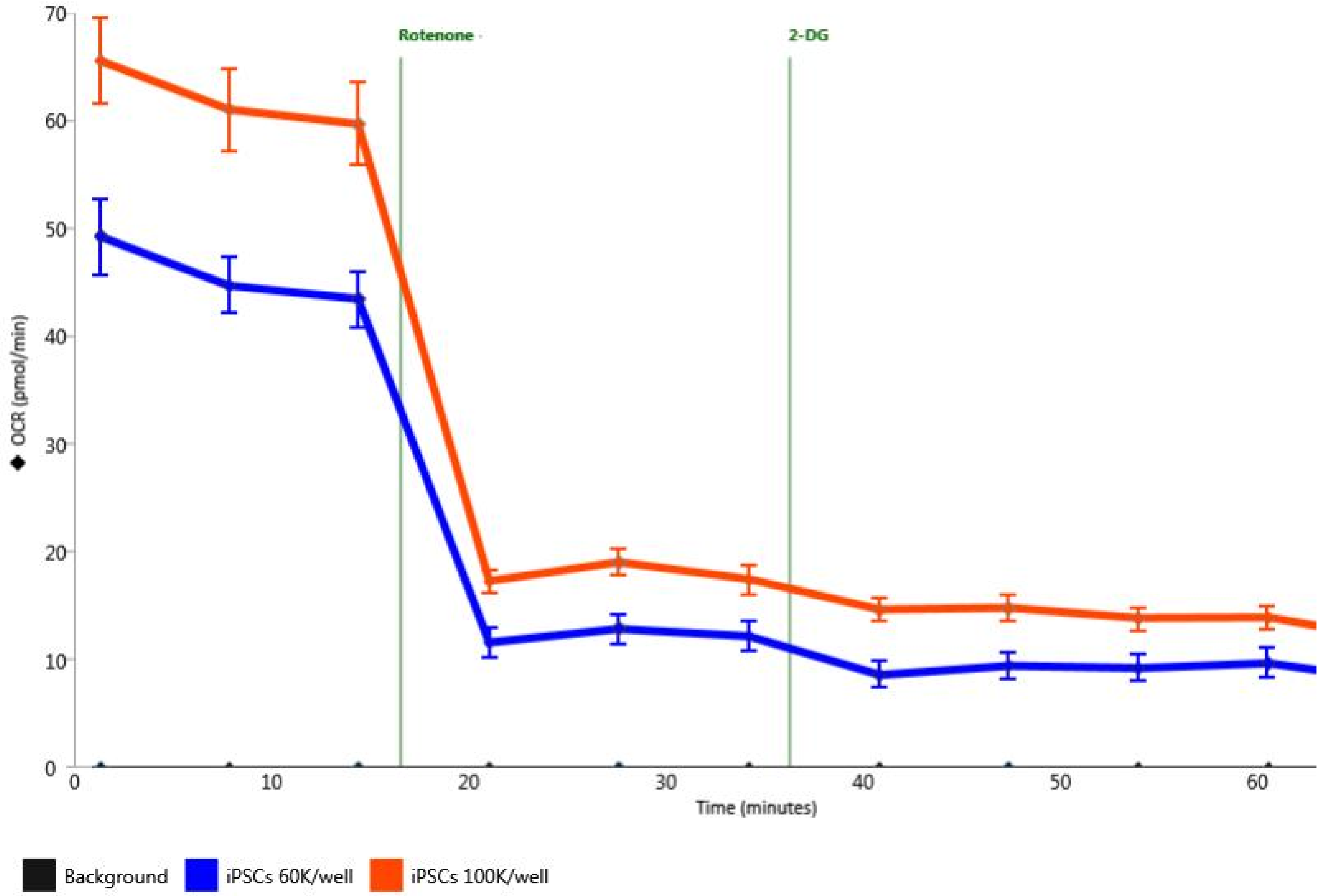
Automatic Calculation of Oxygen Consumption Rate (OCR)



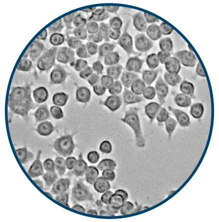
检测特点

Agilent Seahorse XF能量代谢分析技术的特点

实时动态测量

先进的软件&多参数分析

活细胞分析



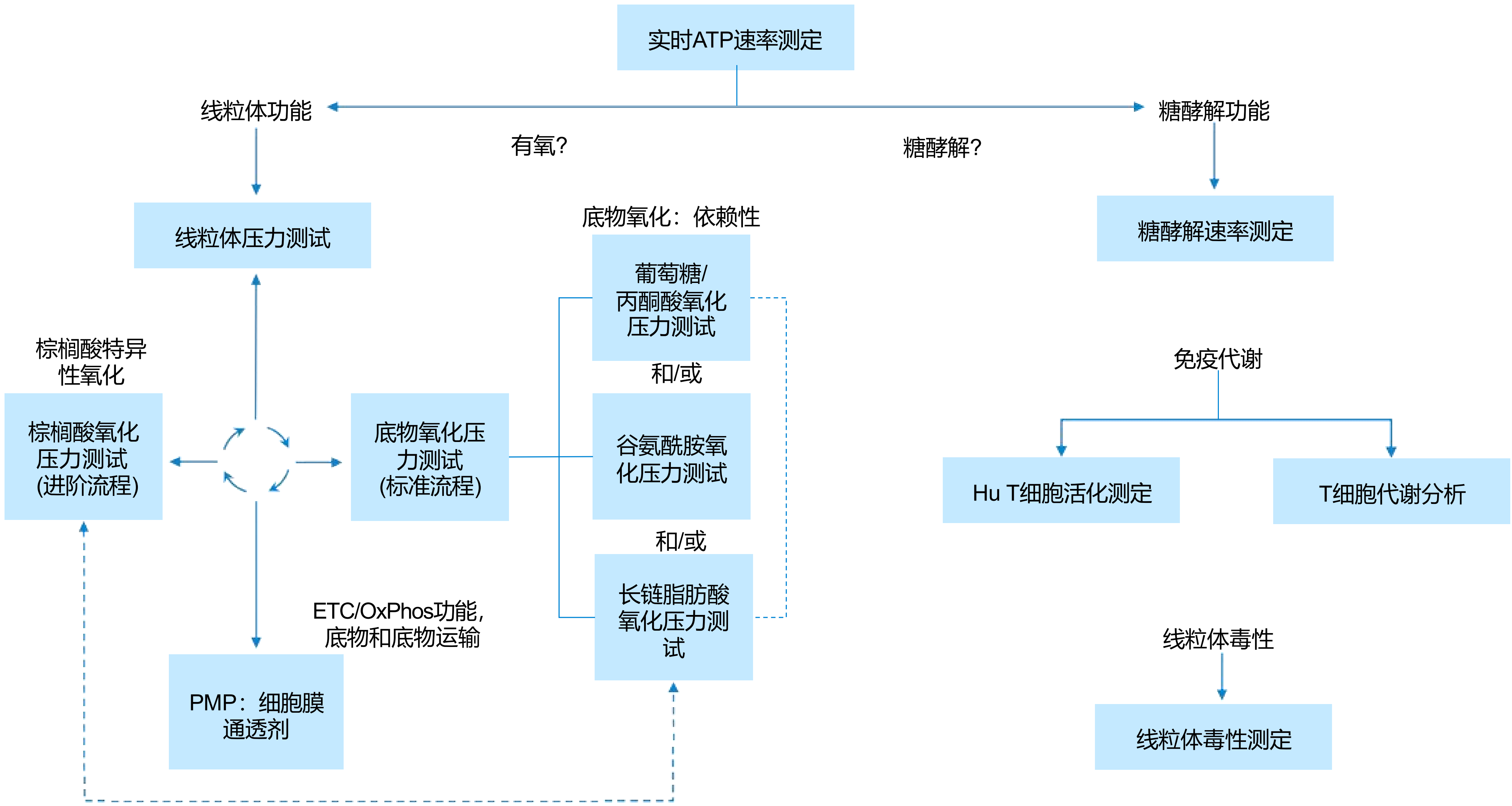
无需标记的H^+ & O_2探针

整合4个加药孔

实时测试药物反应

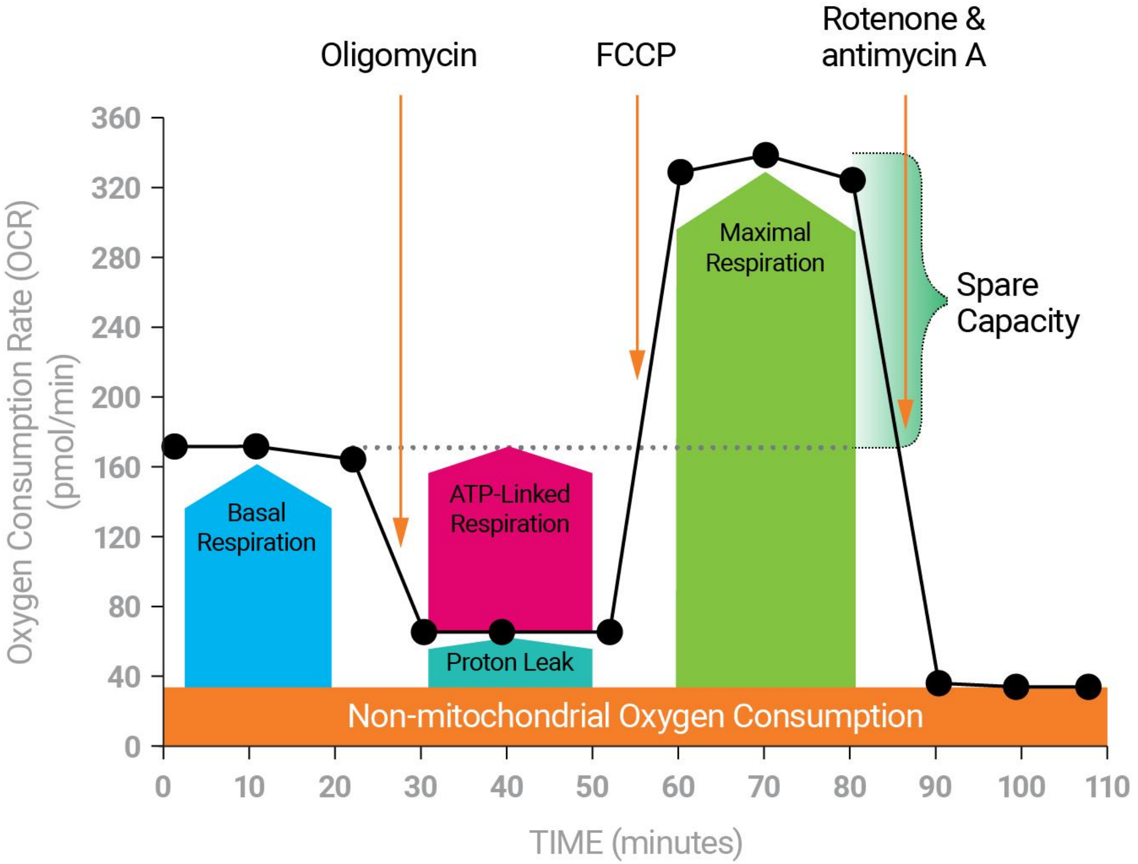
经过验证的即用型试剂盒&试剂

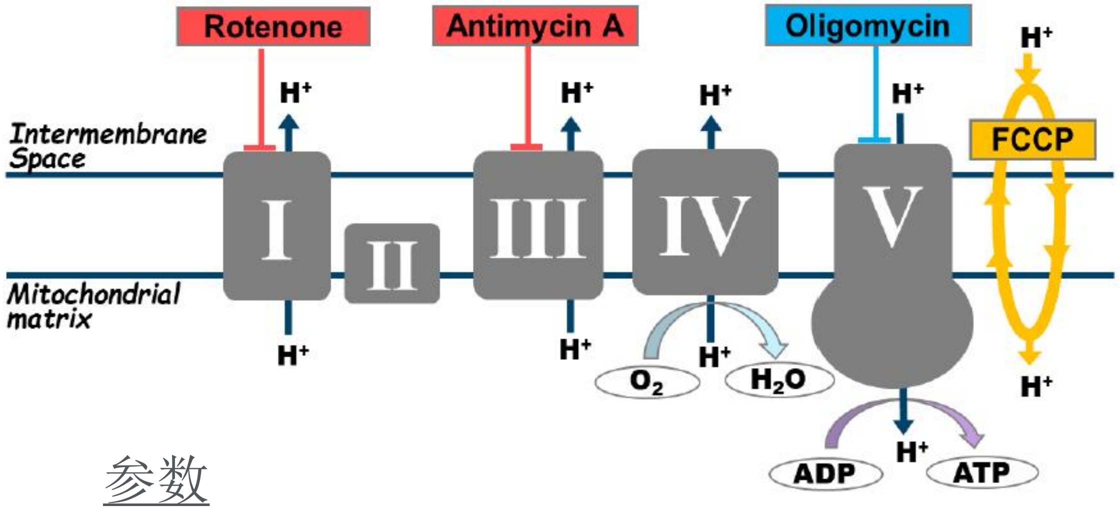
试剂盒介绍


实时ATP速率测定
线粒体功能
有氧?
糖酵解?
糖酵解功能
线粒体压力测试
底物氧化：依赖性
糖酵解速率测定
葡萄糖/
丙酮酸氧化
压力测试
和/或
棕榈酸特异
性氧化
免疫代谢
棕榈酸氧化
压力测试
(进阶流程)
底物氧化压
力测试
(标准流程)
谷氨酰胺氧
化压力测试
Hu T细胞活化测定
T细胞代谢分析
和/或
长链脂肪酸
氧化压力测
试
ETC/OxPhos功能,
底物和底物运输
线粒体毒性
PMP：细胞膜
通透剂
线粒体毒性测定

Agilent Seahorse XF细胞线粒体压力测试

评估细胞线粒体功能

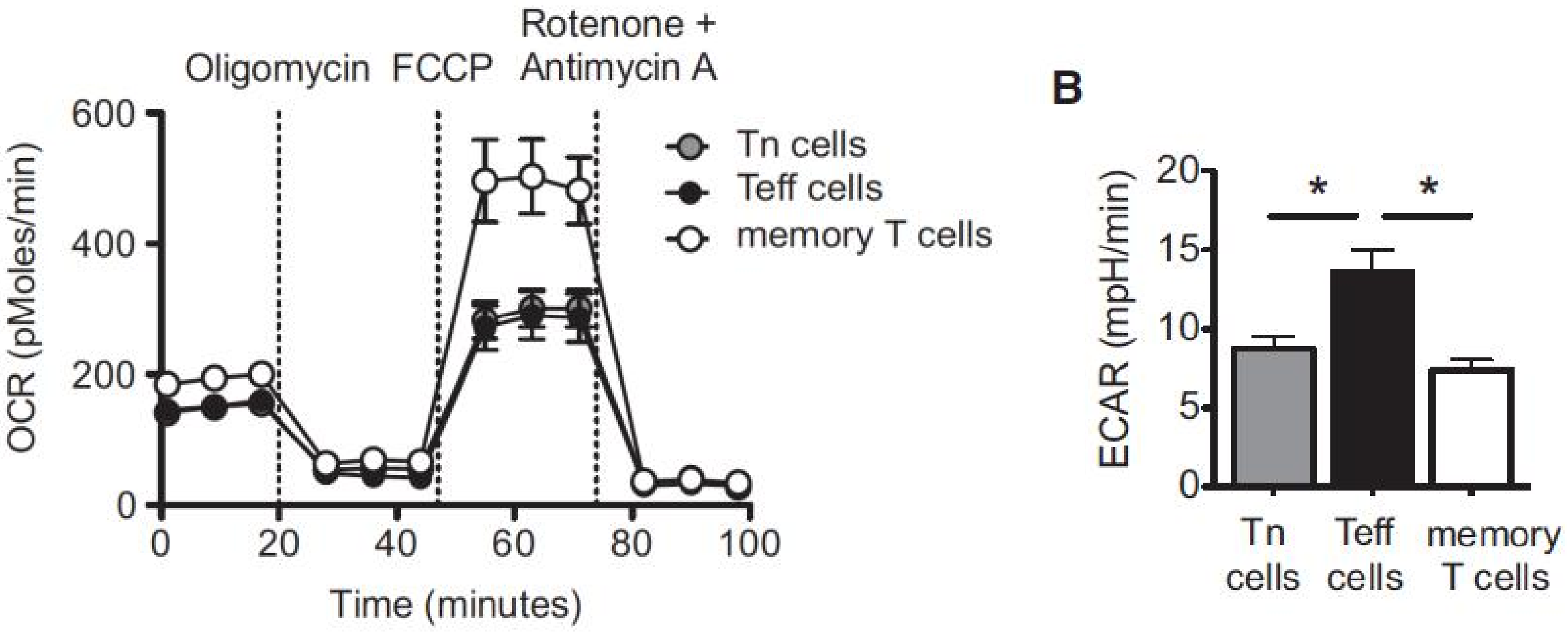




参数

- ❑ 基础呼吸
- ❑ ATP关联呼吸
- ❑ 最大呼吸
- ❑ 备用呼吸能力
- ❑ 质子渗漏

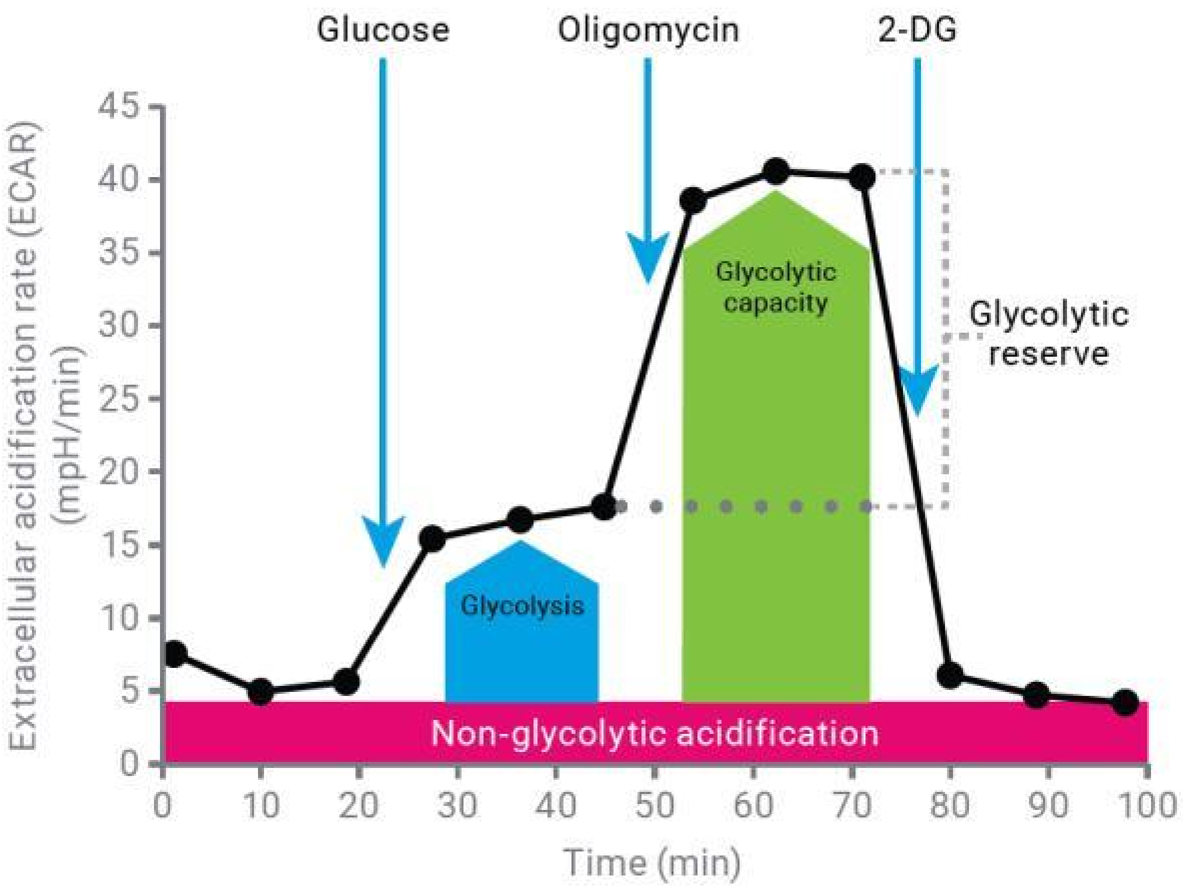
Spare Capacity Affects Memory T Cell Development and Survival



Cell Top 25 Hottest Articles

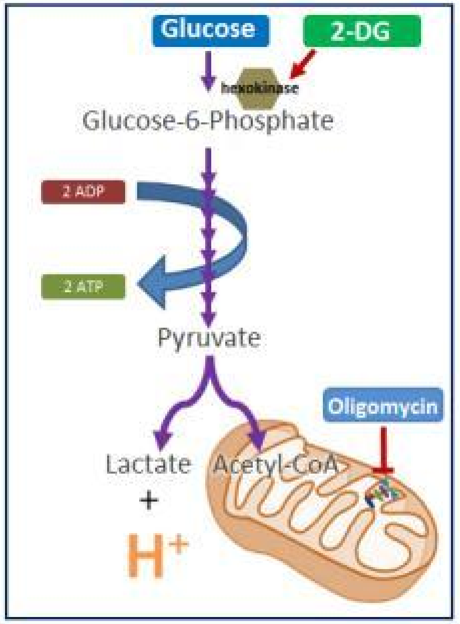
Mitochondrial respiratory capacity is a critical regulator of CD8+ T cell memory development
van der Windt, JW, … and Pearce, EL, Washington School of Medicine. *Immunity* 2012 Jan 27

安捷伦Seahorse XF糖酵解压力测试

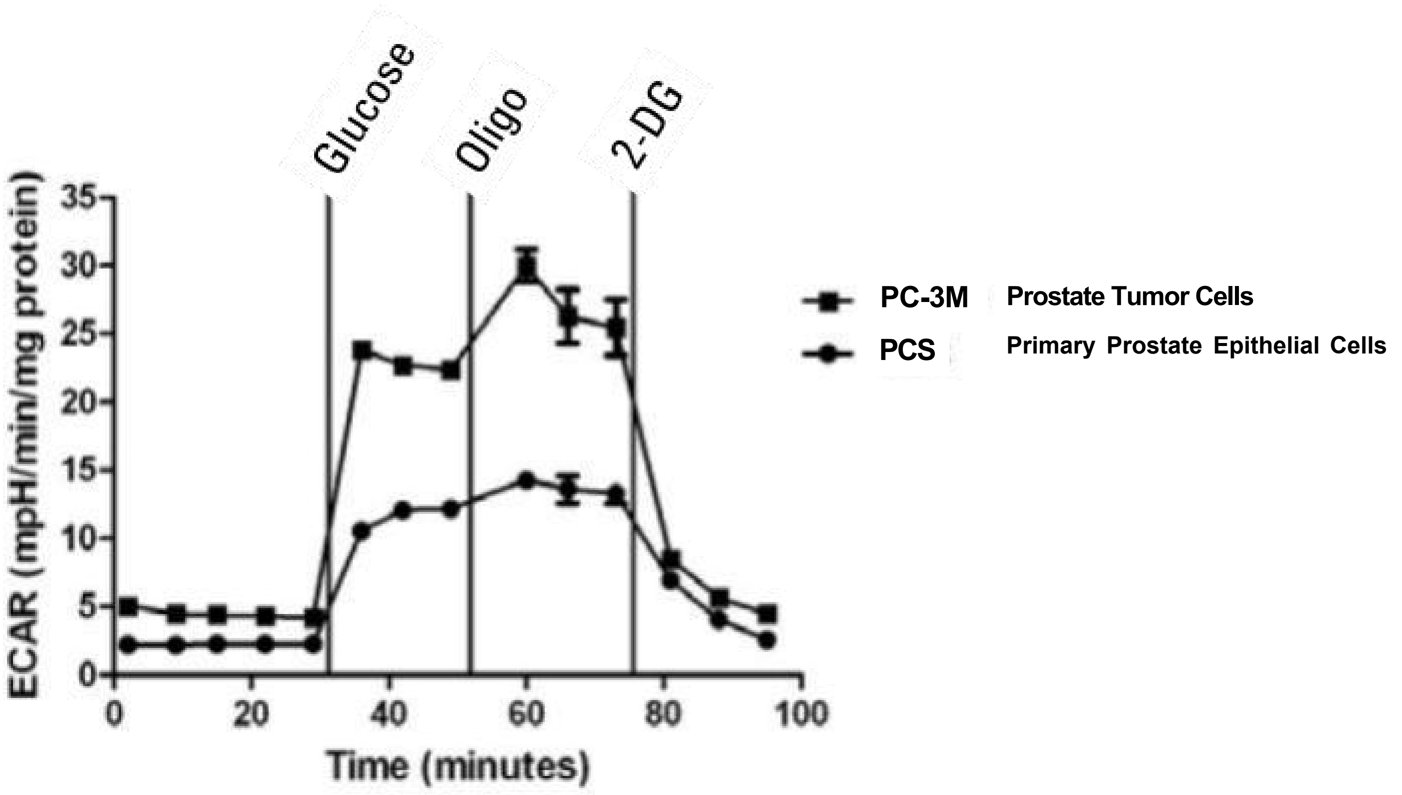


关键参数

- 口 糖酵解
- 口 糖酵解能力
- 口 糖酵解储备

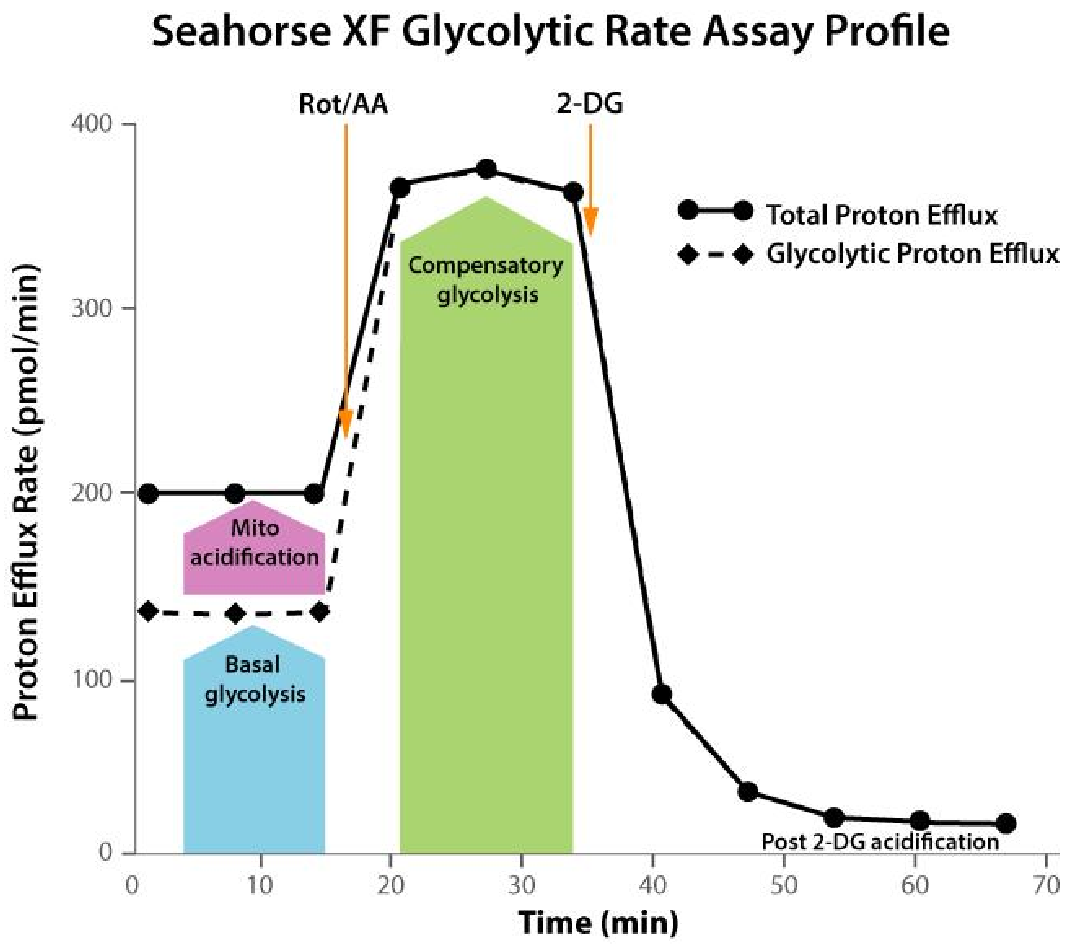


XF Glycolysis Stress Test Measures Glycolytic Phenotype



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糖酵解速率测定



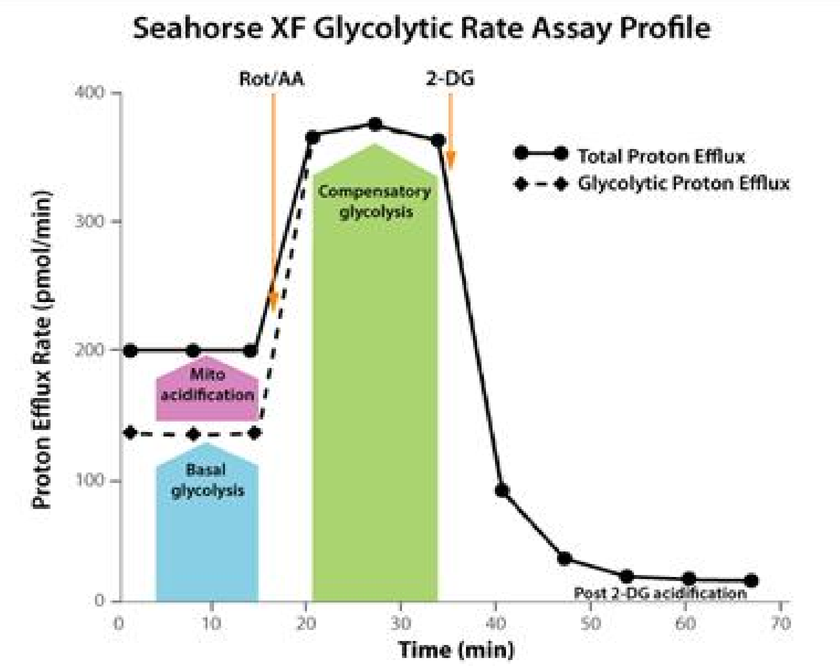
参数

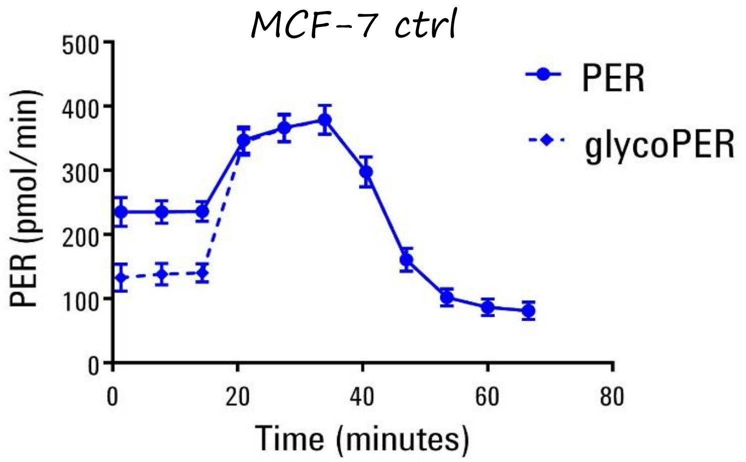
- ❑ 基础糖酵解
- ❑ 补偿性糖酵解

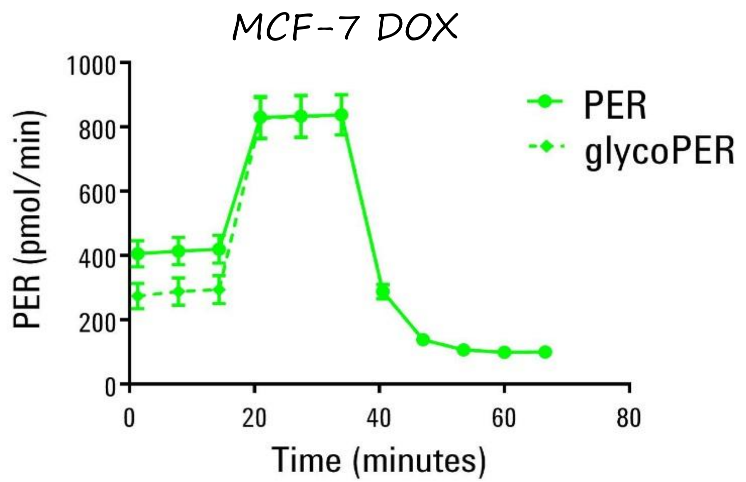
计算CO_2 对细胞外酸化的贡献得到相当于乳酸的糖酵解速率

糖酵解速率测定: Determining the Rate

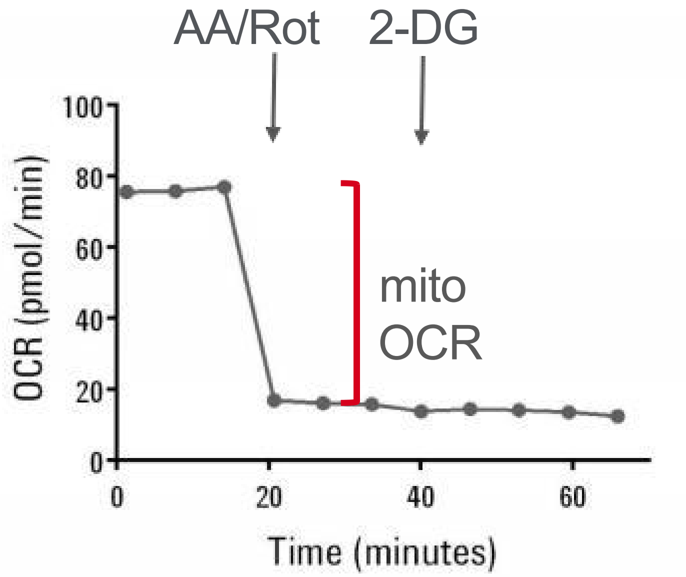
- Convert ECAR to PER (using BF and Kvol)
- Calculate mitoPER (using CCF and OCR)
- Subtract mitoPER from PER to obtain glycoPER

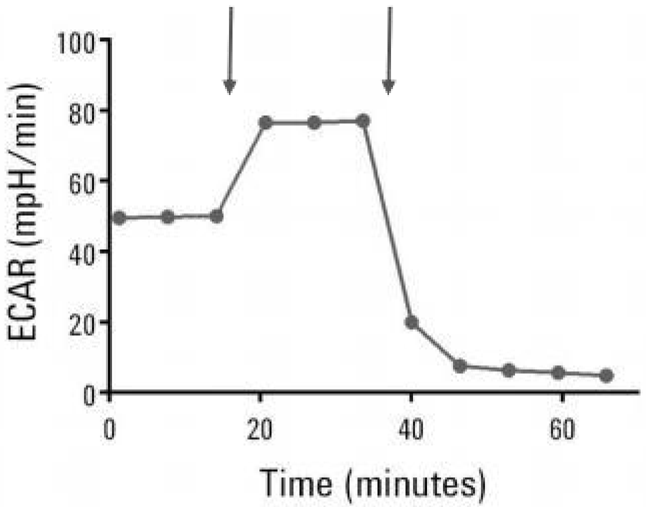






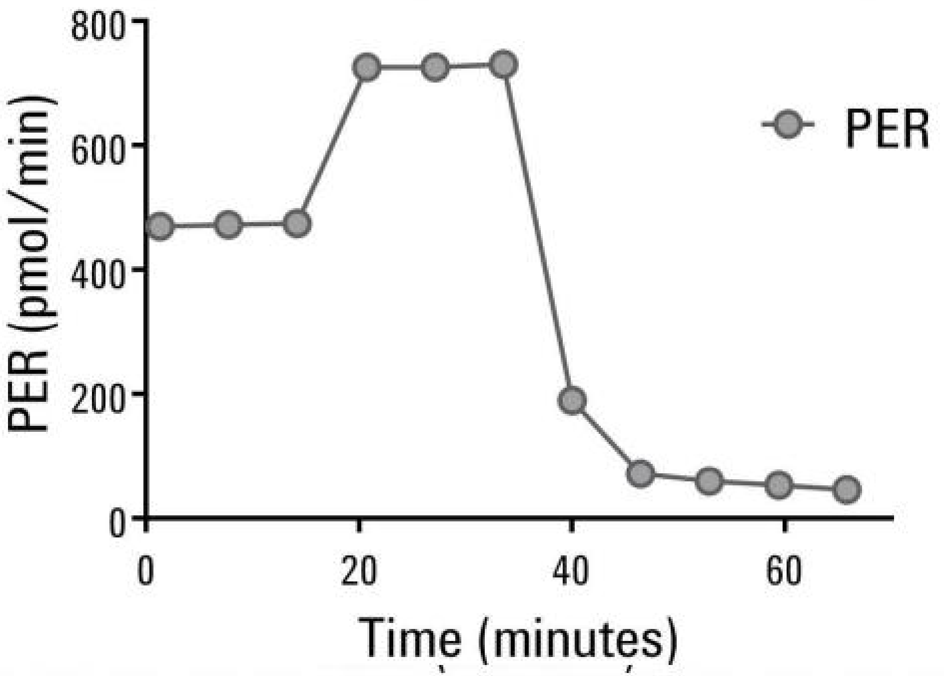
A431 cells: Highly glycolytic





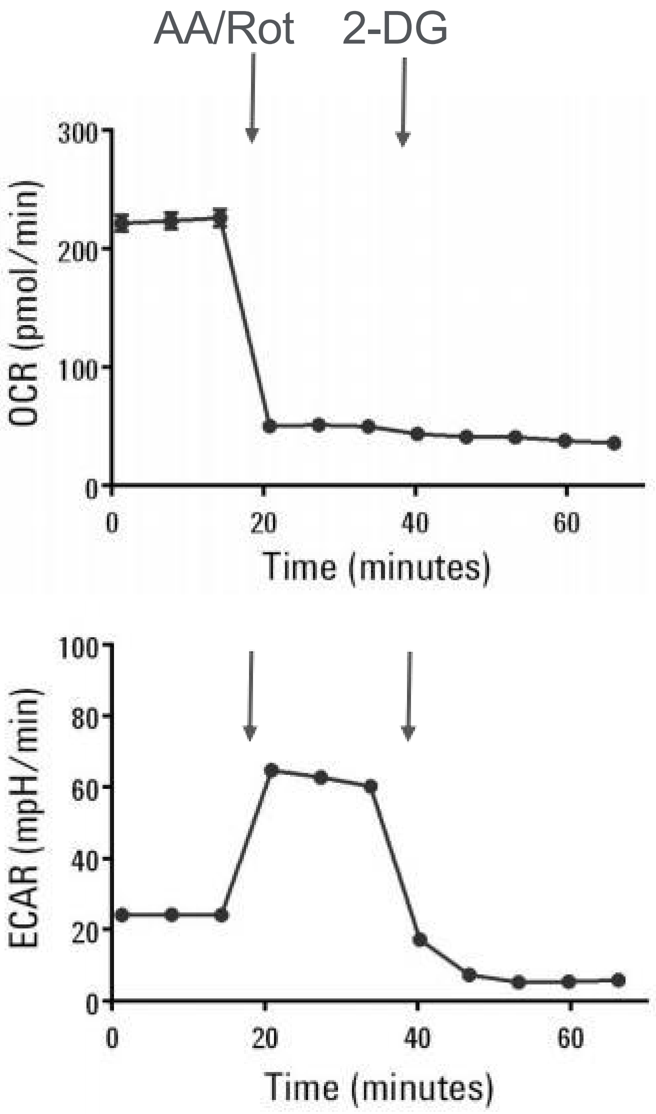
Report Generator

92% glycolytic acidification

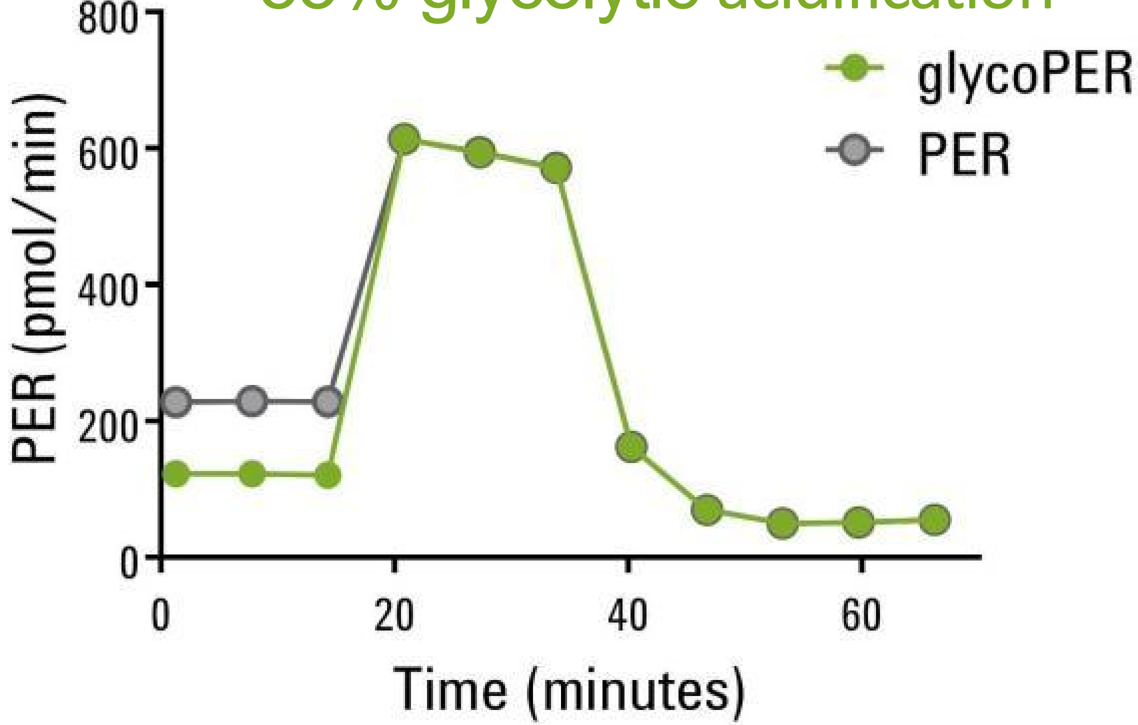


	A431 cells (40K/well)
ECAR (mpH/min)	50 ± 1
PER (pmol/min)	475 ± 4
glycoPER (pmol/min)	**437 ± 4**

Bovine Aortic Endothelial cells (BAEC) : Highly oxidative

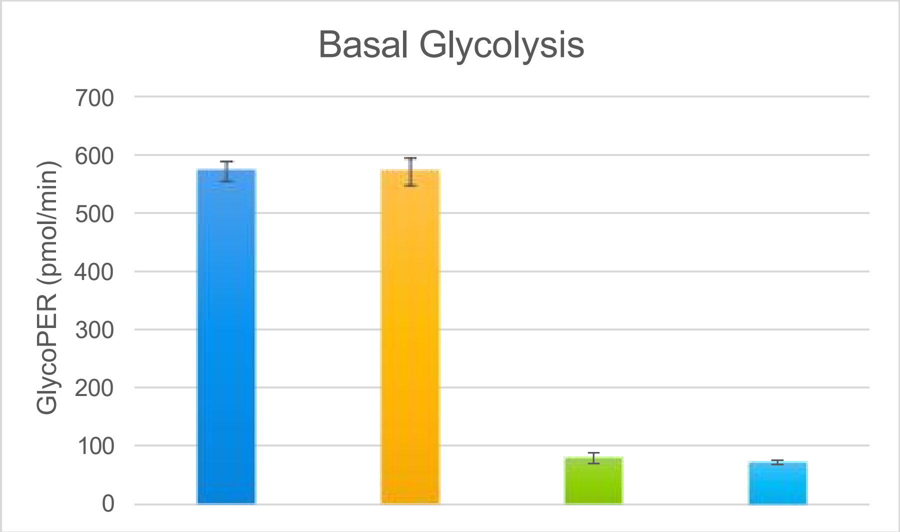


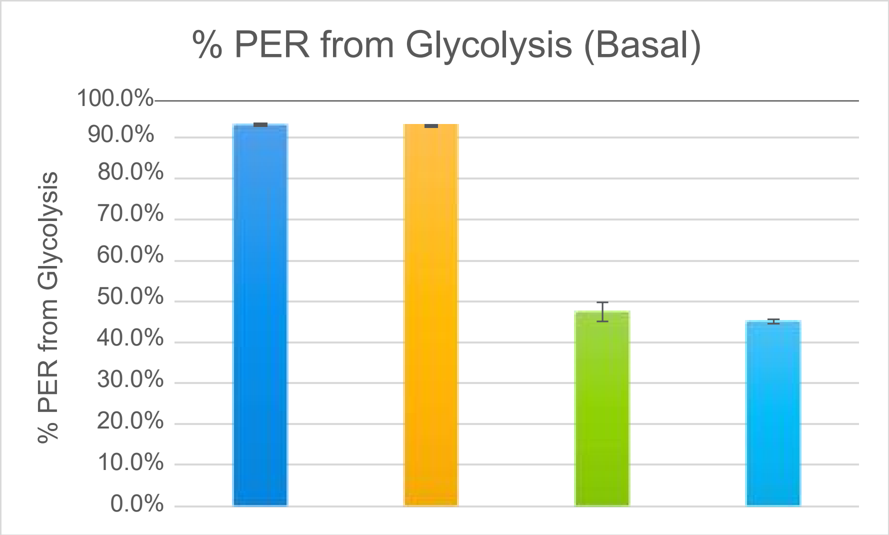
53% glycolytic acidification

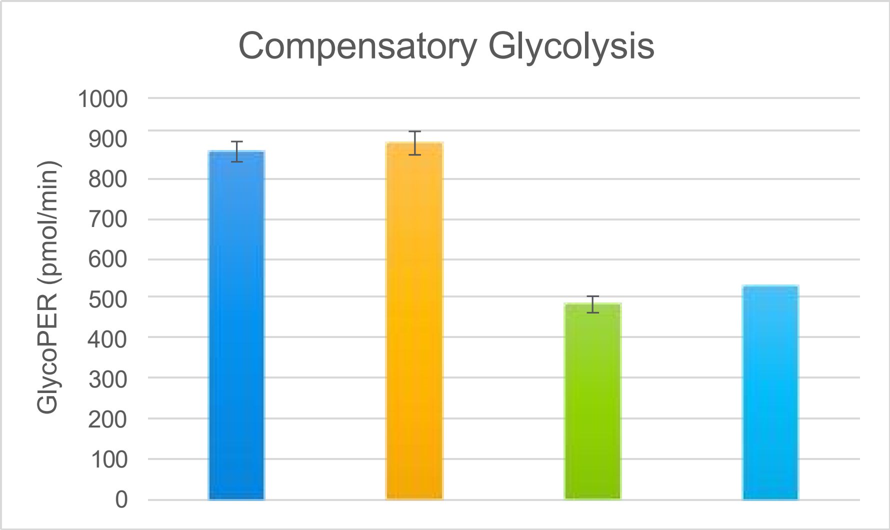


	BAEC (50K/well)
ECAR (mpH/min)	24 ± 1
PER (pmol/min)	220 ± 9
glycoPER (pmol/min)	**117 ± 6**

Assay Outputs : Report Generator







Parameter
Basal Glycolysis (pmol/min)
Basal Proton Efflux Rate (PER) (pmol/min)
% PER from Glycolysis (Basal)
Compensatory Glycolysis (pmol/min)
Basal mitoOCR/glycoPER
Post 2-DG Acidification (pmol/min)

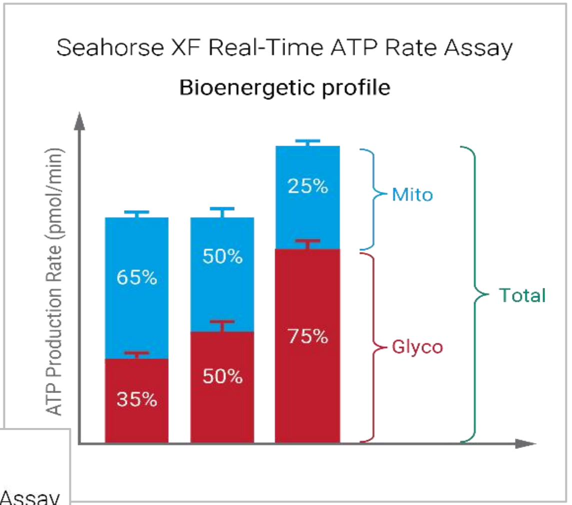
安捷伦Seahorse XF实时ATP速率测定

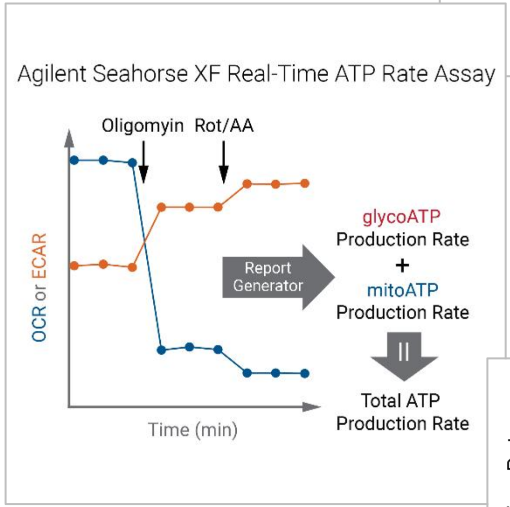
实时同时测量活细胞线粒体呼吸和糖酵解ATP产生速率

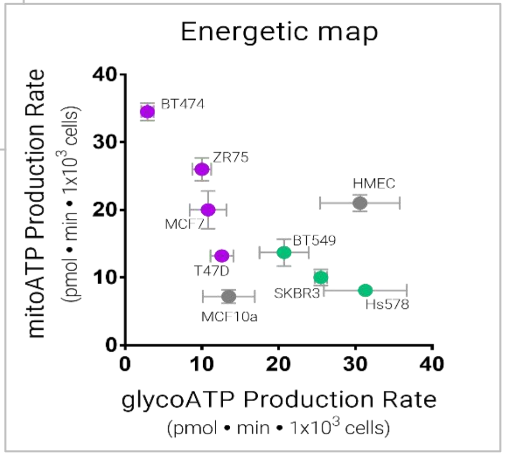
1. 测量细胞功能的新方法。

2. 提供有关ATP动力学的新视角，为细胞表型和功能研究提供新的见解。

3. 量化代谢转换并鉴定通路功能障碍。





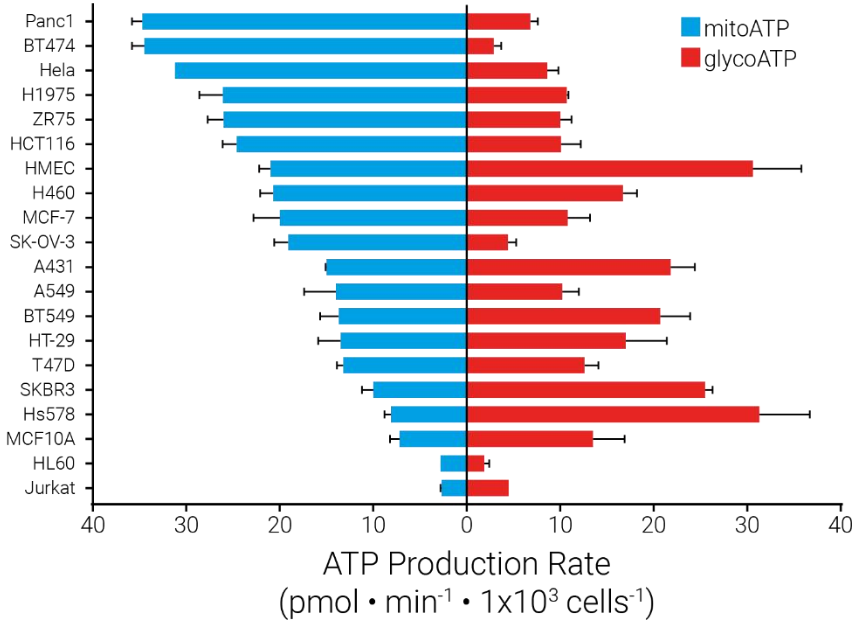


Bioenergetic phenotype reveals liabilities in cancer

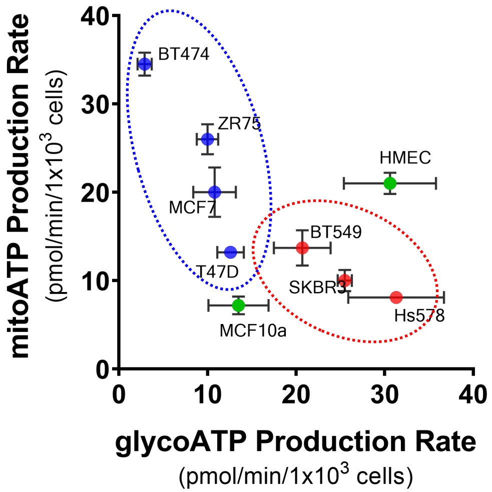
XF Real-Time ATP Rate Assay

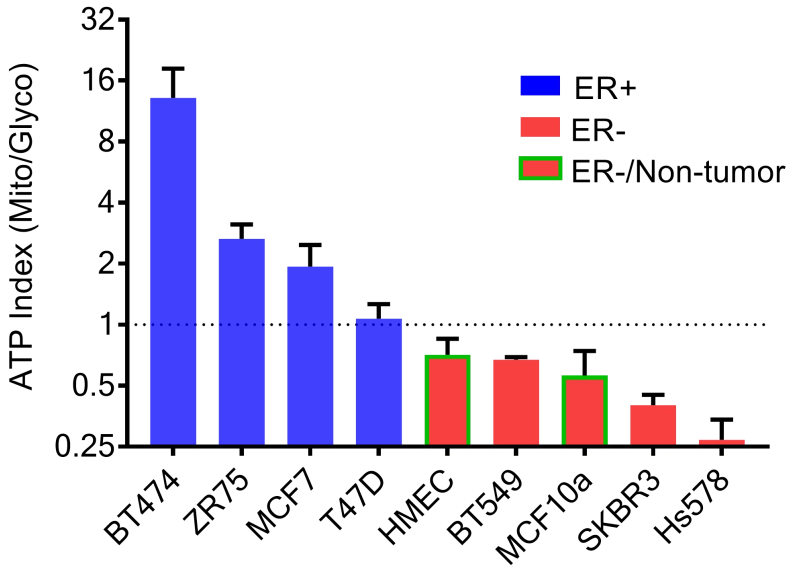
雌激素受体阳性 (ER+) 乳腺癌细胞比ER-或对照细胞更依赖氧化代谢

Metabolic diversity in a panel of cancer cell lines

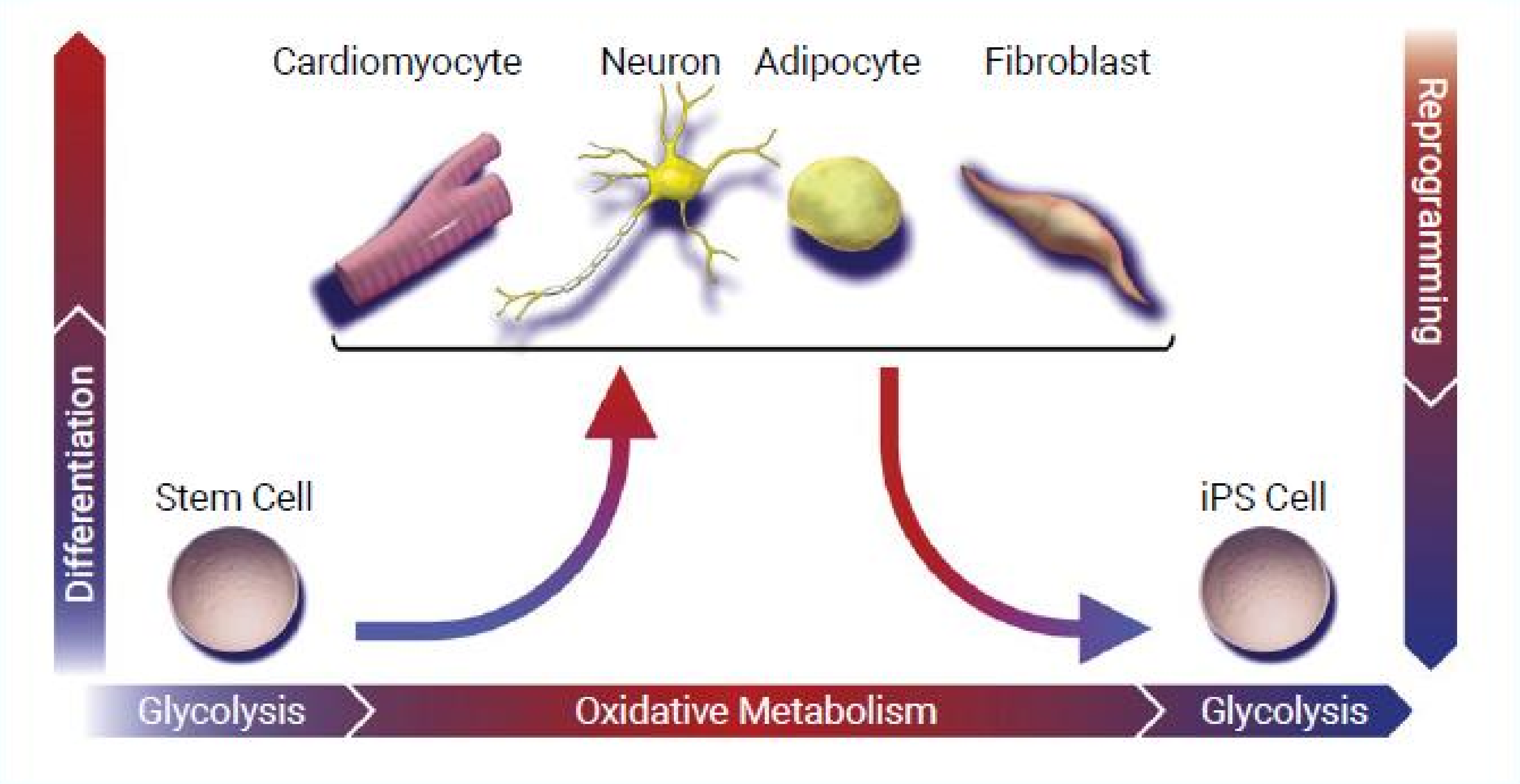


Invasive ER+ breast cancer cells are characterized by a more oxidative metabolic phenotype





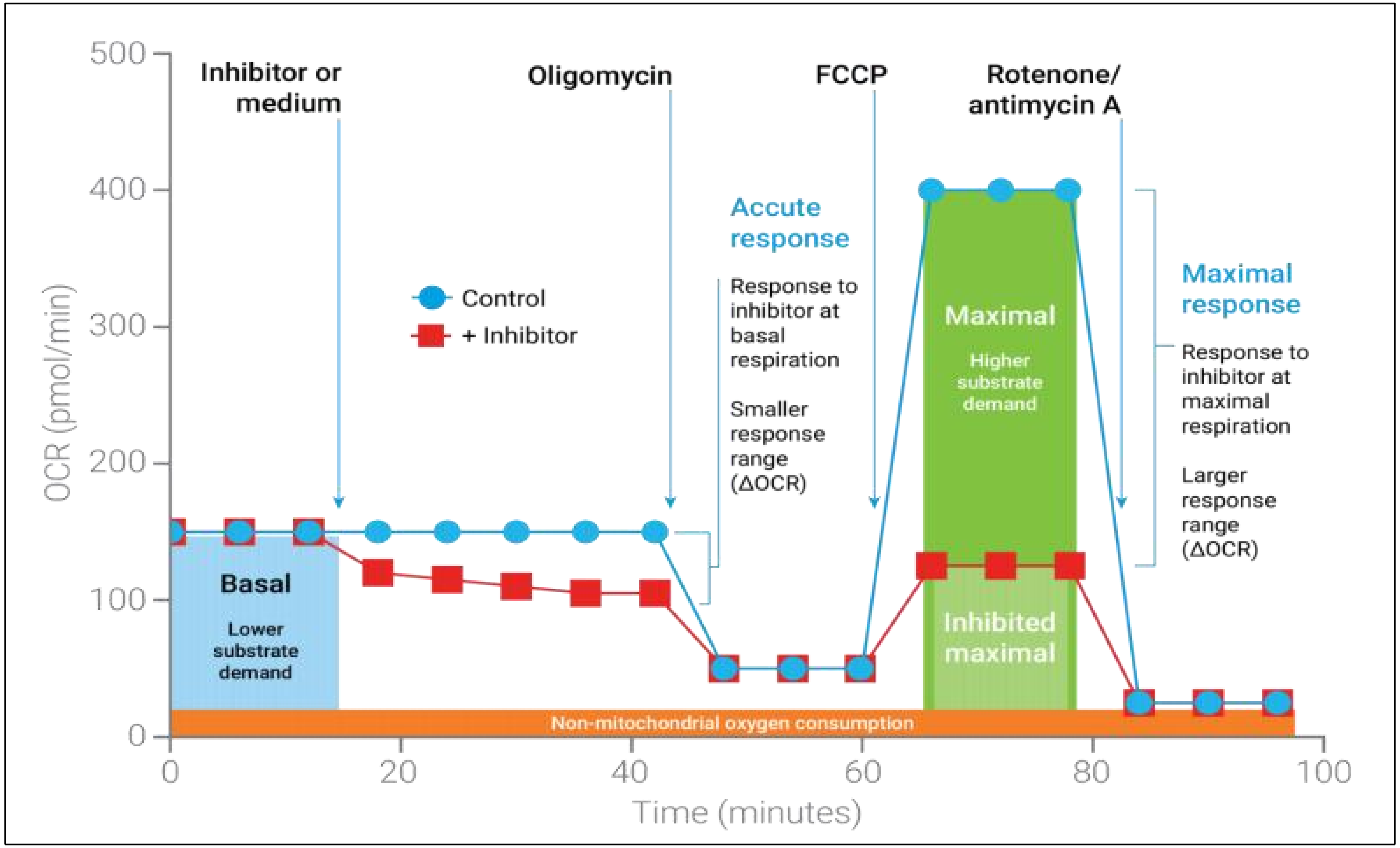
Agilent Seahorse Poster # 3487 AACR 2018



Metabolic plasticity in stem cell homeostasis and differentiation.
Folmes, C. D., *et al.* Cell Stem Cell. 2012. 11: 596-606.

安捷伦Seahorse XF底物氧化压力测试

探索驱动线粒体功能的底物氧化途径



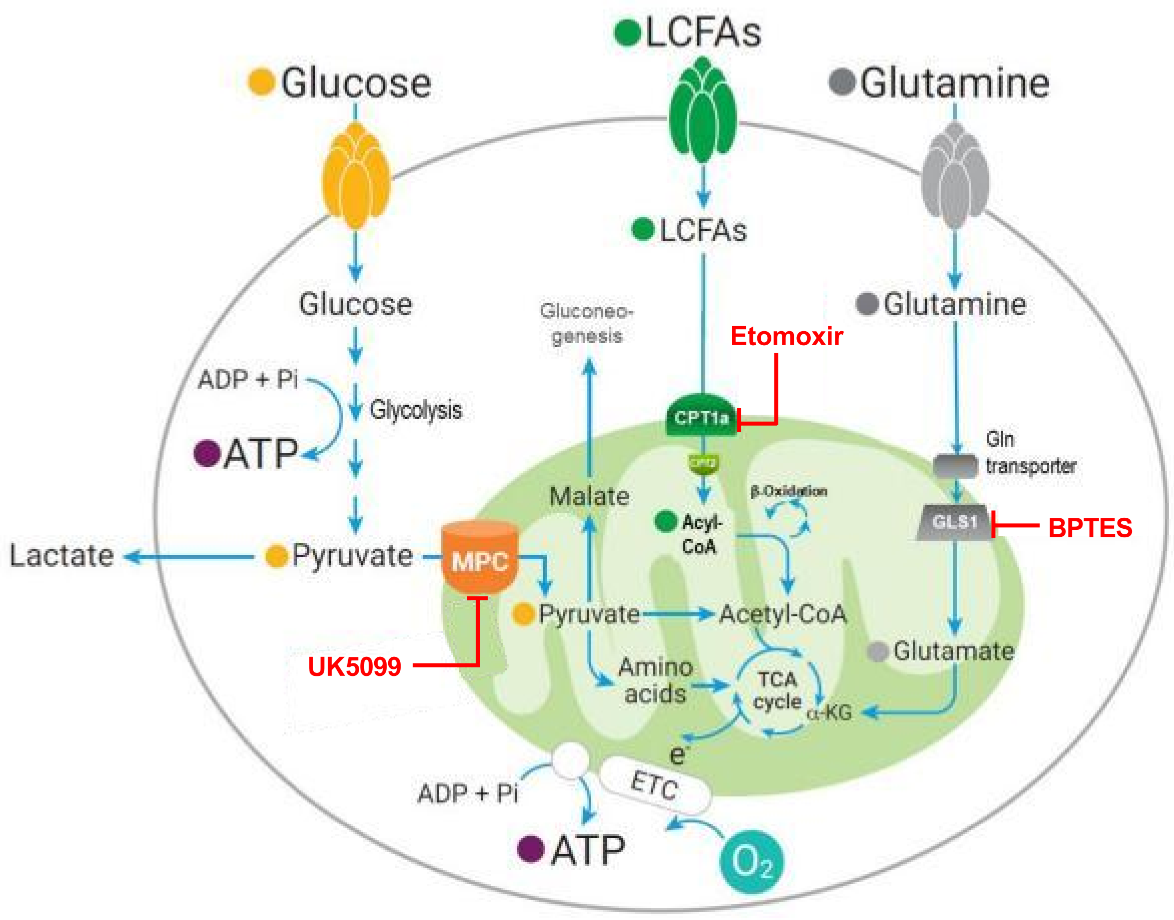
关键参数

- 口 基础OCR
- 口 急性响应
- 口 *最大OCR*

XF底物氧化压力测试是基于已被广泛认可的用于测量线粒体功能的XF细胞线粒体压力测试，使用优化的通路特异性抑制剂来揭示细胞氧化三种主要底物的能力。

评估特定底物氧化：工作原理？

使用通路特异性抑制剂来评估特定底物的氧化



UK5099 (抑制葡萄糖氧化)

线粒体丙酮酸载体(MPC)的抑制剂， MPC将丙酮酸运输到线粒体中

Etomoxir (抑制长链脂肪酸氧化)

肉碱棕榈酰转移酶- 1 (CPT- 1)的抑制剂， CPT- 1对于长链脂肪酸从细胞质基质转运到线粒体中氧化至关重要

(注: 浓度> 4 μM 会对线粒体呼吸产生脱靶效应)

BPTES (抑制谷氨酰胺氧化)

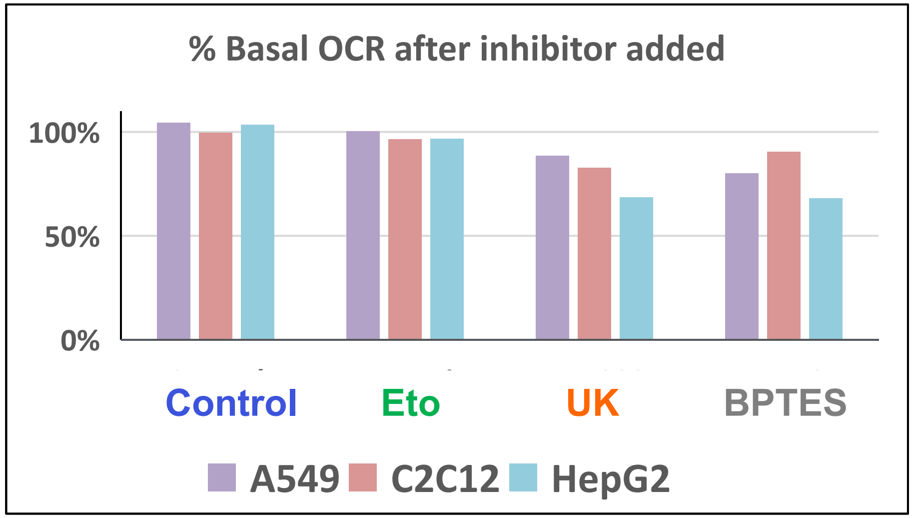
谷氨酰胺酶(GLS1)的抑制剂。 GLS1将谷氨酰胺转变为谷氨酸，谷氨酸再转变为α-酮戊二酸，通过TCA循环氧化

(注: BPTES不抑制GLS2)

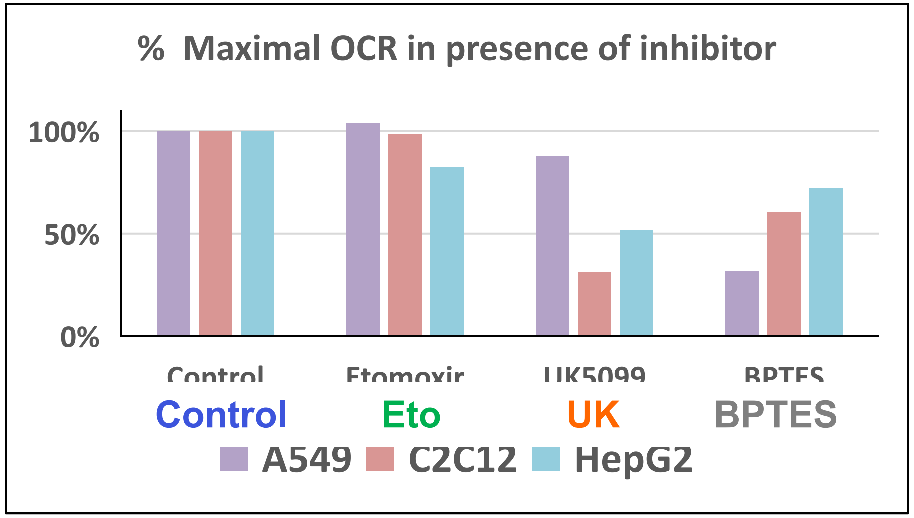
使用XF葡萄糖/丙酮酸、长链脂肪酸和谷氨酰胺氧化压力测试比较A549、C2C12和 HepG2细胞的底物氧化

Acute Injection	Control (media)	BPTES	Etomoxir (Eto)	UK5099 (UK)
Substrate Oxidation Impacted	-	Glutamine	LCFAs	Glucose/Pyruvate

Relative Substrate Oxidation Patterns among A549, C2C12 and HepG2 Cells



% Basal OCR	LCFAs (Etomoxir)	Glucose/Pyruvate (UK5099)	Glutamine (BPTES)
A549	100%	88%	80%
C2C12	96%	83%	90%
HepG2	97%	69%	68%

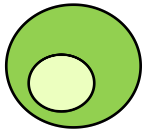


% Maximal OCR	LCFAs (Etomoxir)	Glucose/Pyruvate (UK5099)	Glutamine (BPTES)
A549	104%	88%	32%
C2C12	97%	31%	60%
HepG2	82%	52%	72%

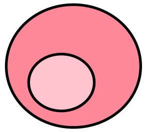
示例 – XF葡萄糖/丙酮酸氧化压力测试

“我想比较细胞A和细胞B在葡萄糖氧化方面的情况。也就是说，不同细胞的线粒体对葡萄糖的需求是否存在差异？ ”

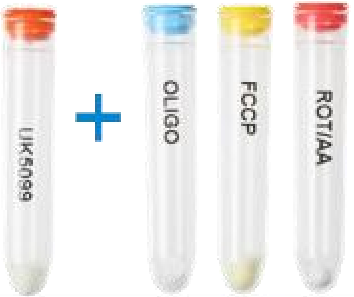
Cell Type A



Cell Type B



XF Glucose/Pyruvate
Oxidation Stress Test



定义：

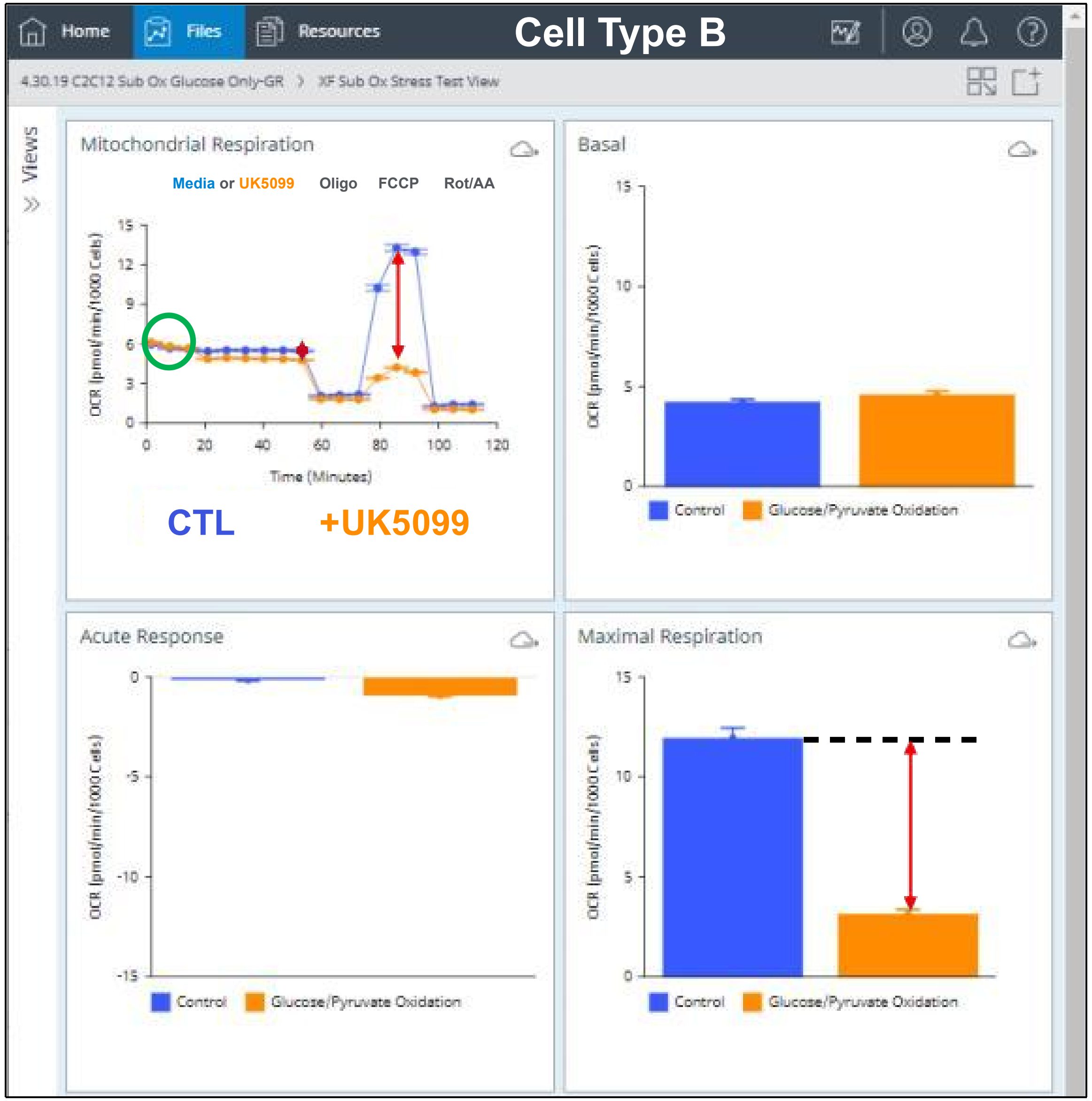
干预:
在XF检测前进行 (慢性或急性干预)。

急性注射:
加入抑制剂(通过加药孔A)，然后加入Oligomycin, FCCP和Rot/AA。

标准底物氧化压力测试实验模板

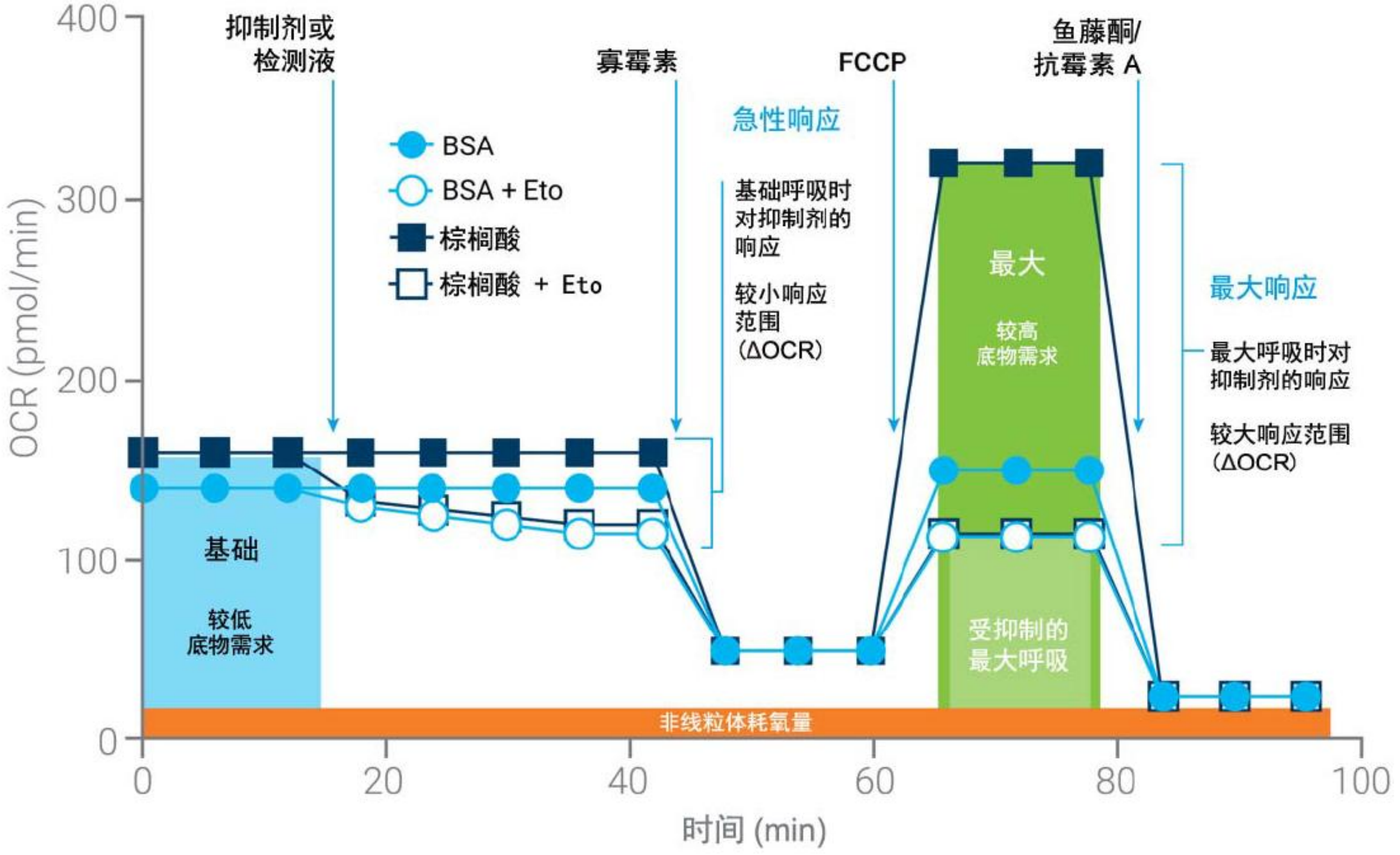
慢性干预* 如基因操作、慢性药物处理 → 细胞接种 → **急性干预*** (预处理) 如化合物处理 → 葡萄糖/丙酮酸氧化压力测试 **Basal > UK5099 > Oligo > FCCP > Rot/AA** 检测液 = **DMEM +** 葡萄糖、 丙酮酸钠、 谷氨酰胺 → **Sub Ox Analytics > Basal, Acute and Maximal OCR**

XF葡萄糖/丙酮酸氧化压力测试 – 细胞Avs. 细胞B



安捷伦Seahorse XF棕榈酸氧化压力测试

Profile of the respiration parameters relevant for Palmitate/LCFA demand



关键参数

- 口 基础OCR
- 口 急性响应
- 口 *最大OCR*

棕榈酸氧化会受到什么影响:

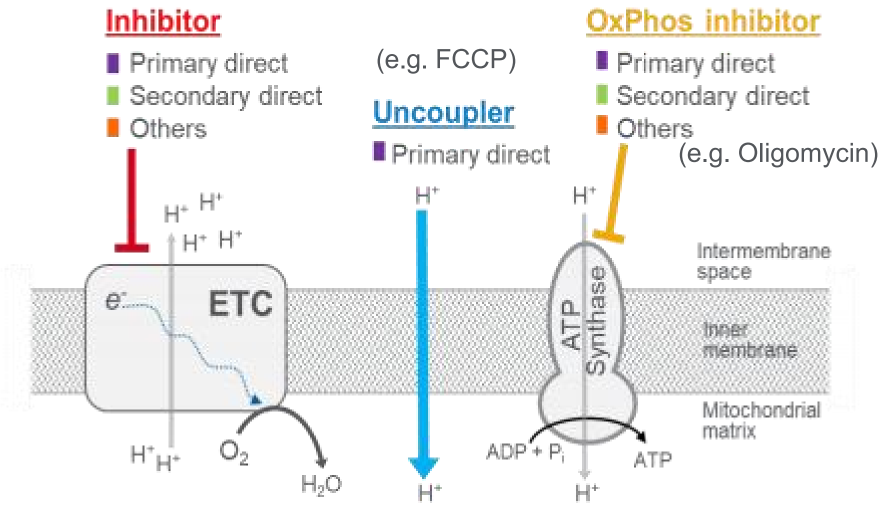
- 口 如果对细胞进行基因操作?
- 口 如果用化合物处理细胞?

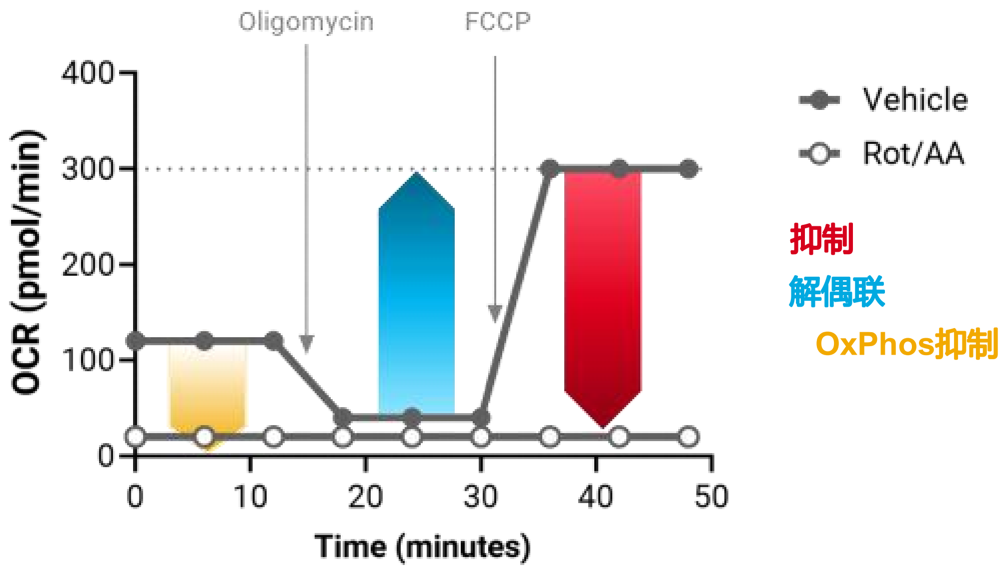
存在或不存 在 Etomoxir 时，连续添加化合物，测定基础呼吸、对 Etomoxir 的急性响应以及最大呼吸。

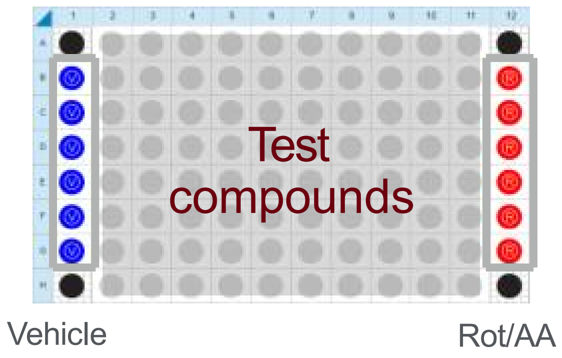
虽然在基础条件下可以检测到较小的变化，即急性响应，但在高底物需求条件下 （如FCCP），往往会出现更大的响应，从而显示出细胞氧化棕榈酸能力的差异

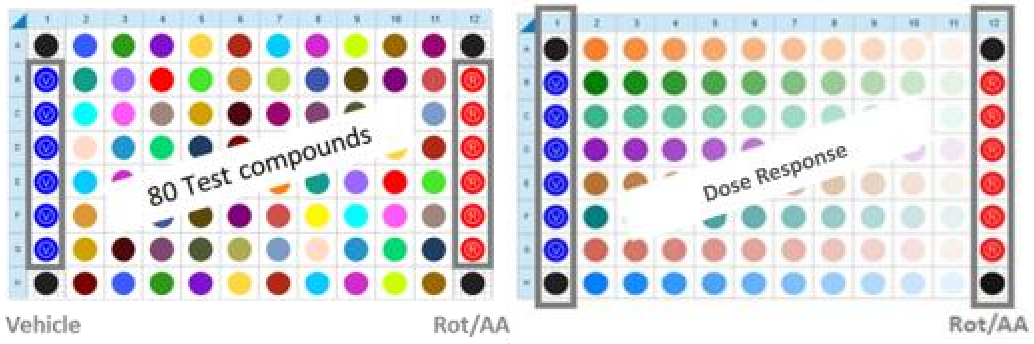
安捷伦Seahorse XF线粒体毒性测定

评估药物诱导的线粒体毒性



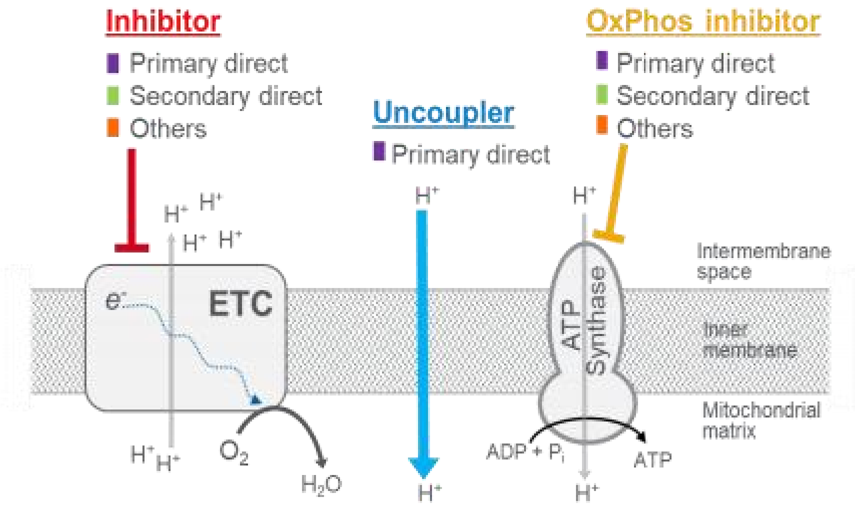


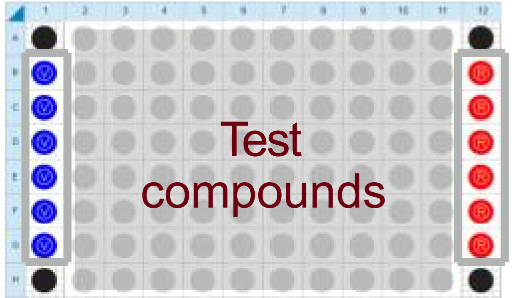


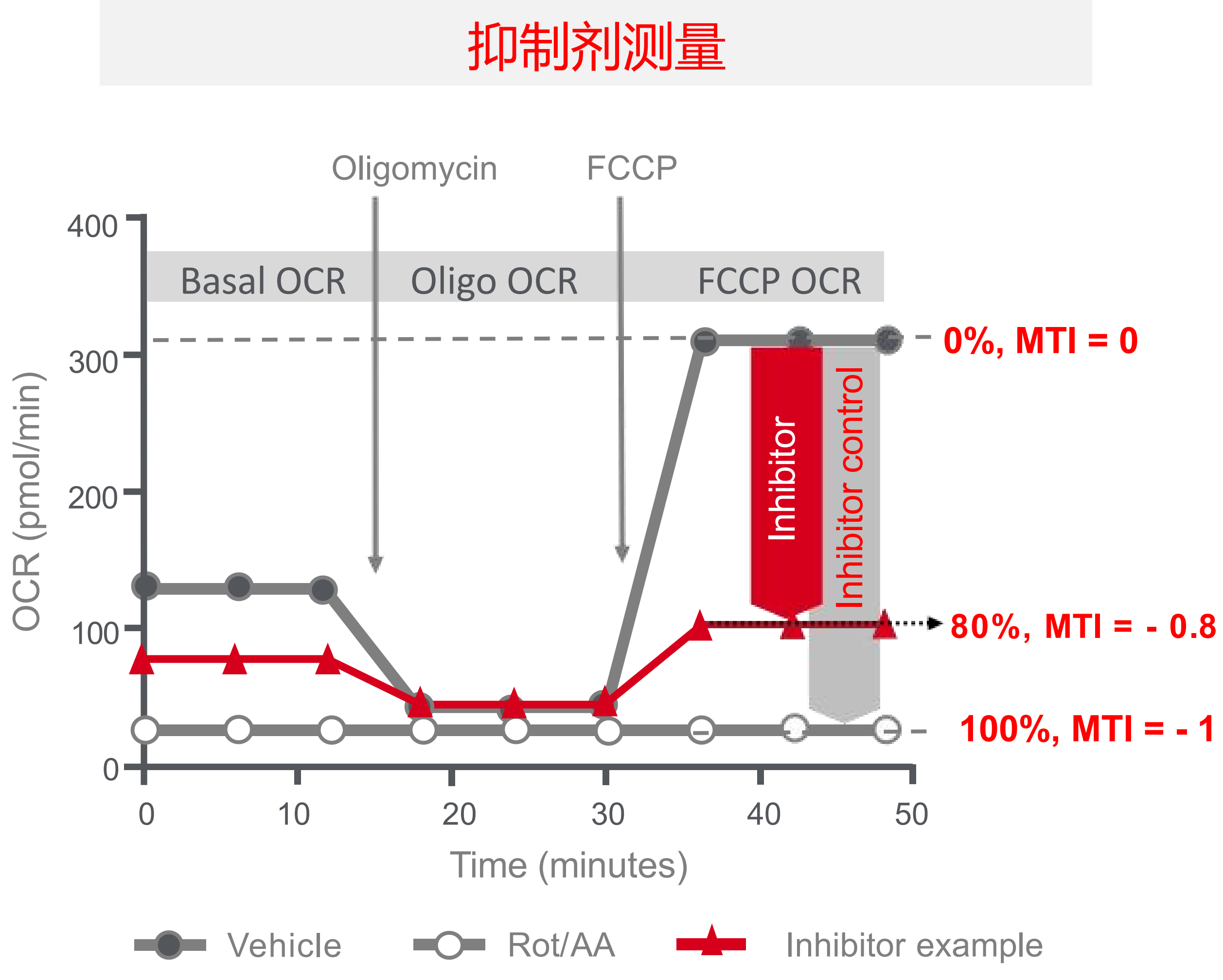


Simplified Template Design

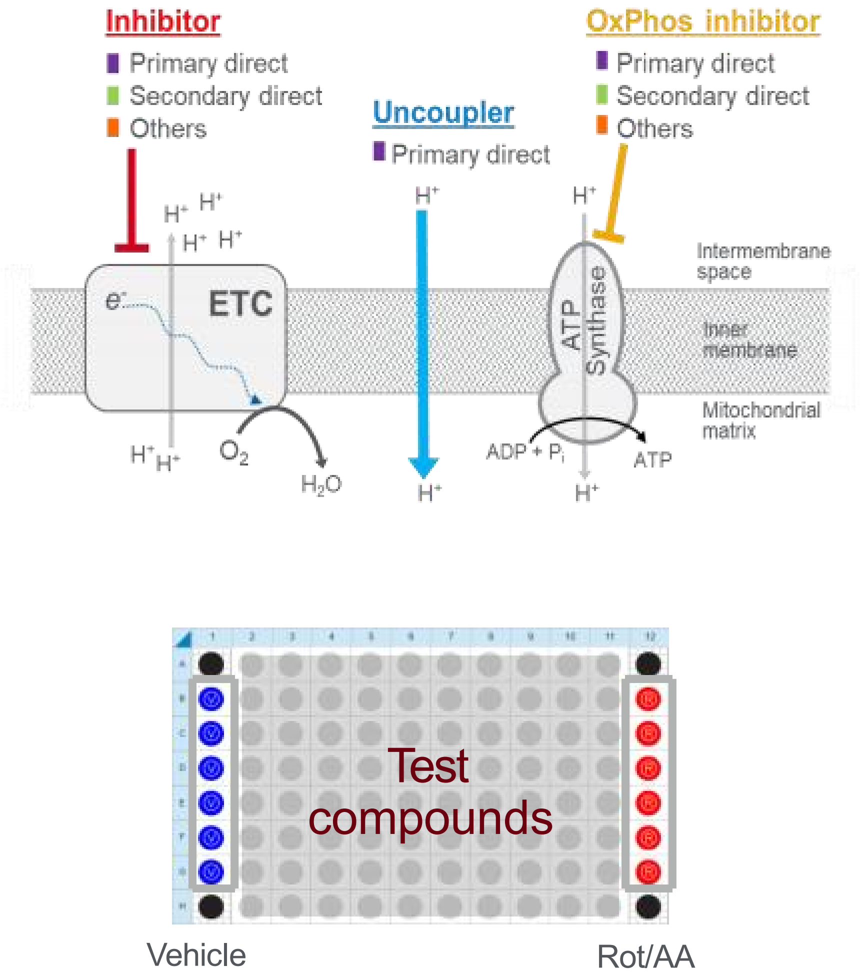
Seahorse XF Mito Tox Assay Solution: MTI for Inhibitor



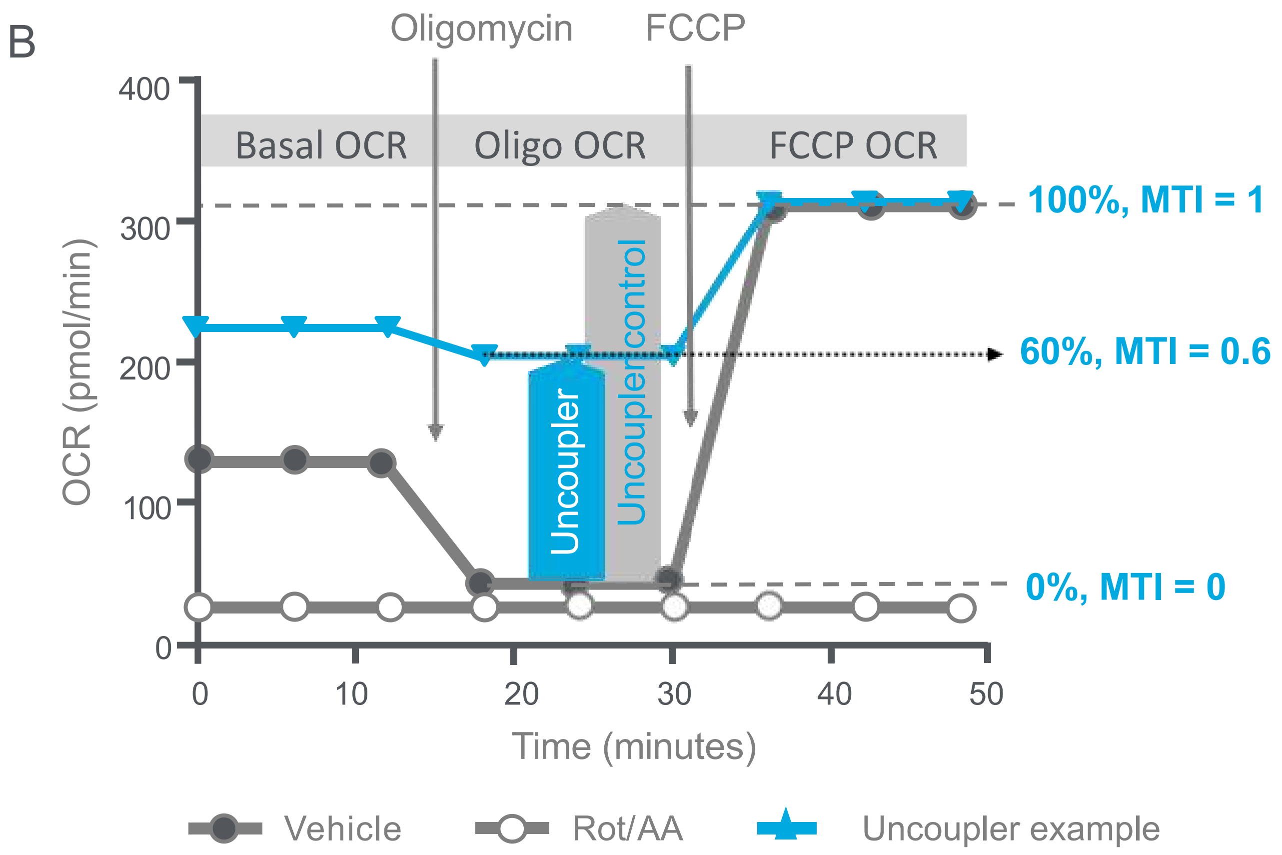




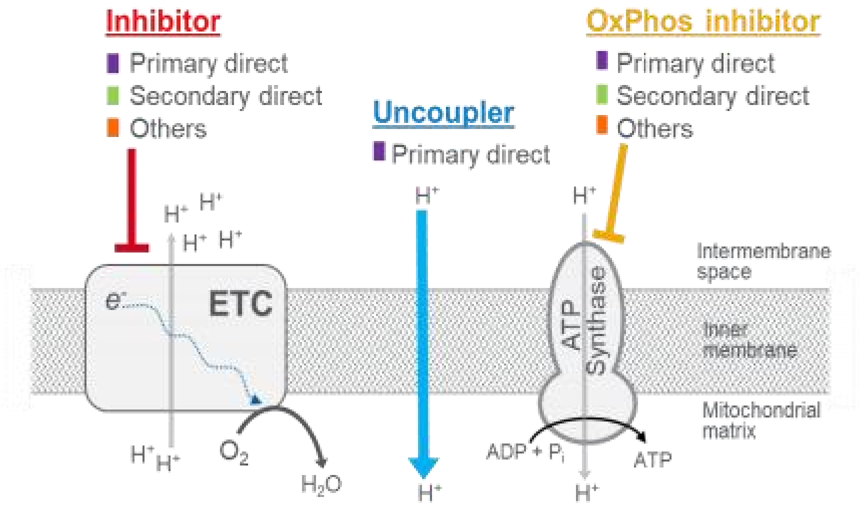
Seahorse XF Mito Tox Assay Solution: MTI for Uncoupler

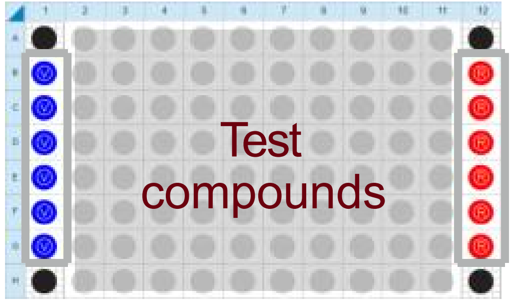


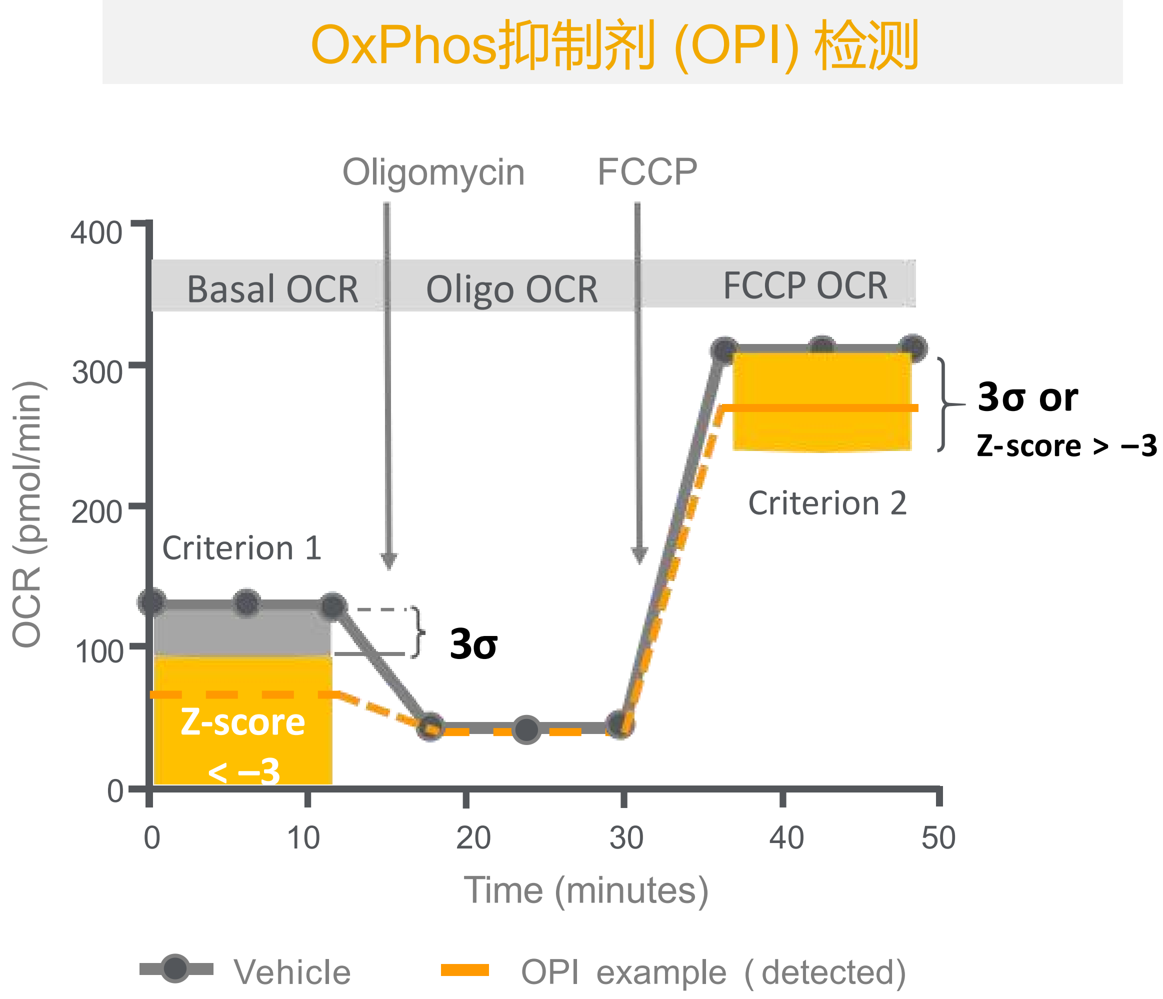
解偶联剂测量



Seahorse XF Mito Tox Assay Solution: Detection of OPIs



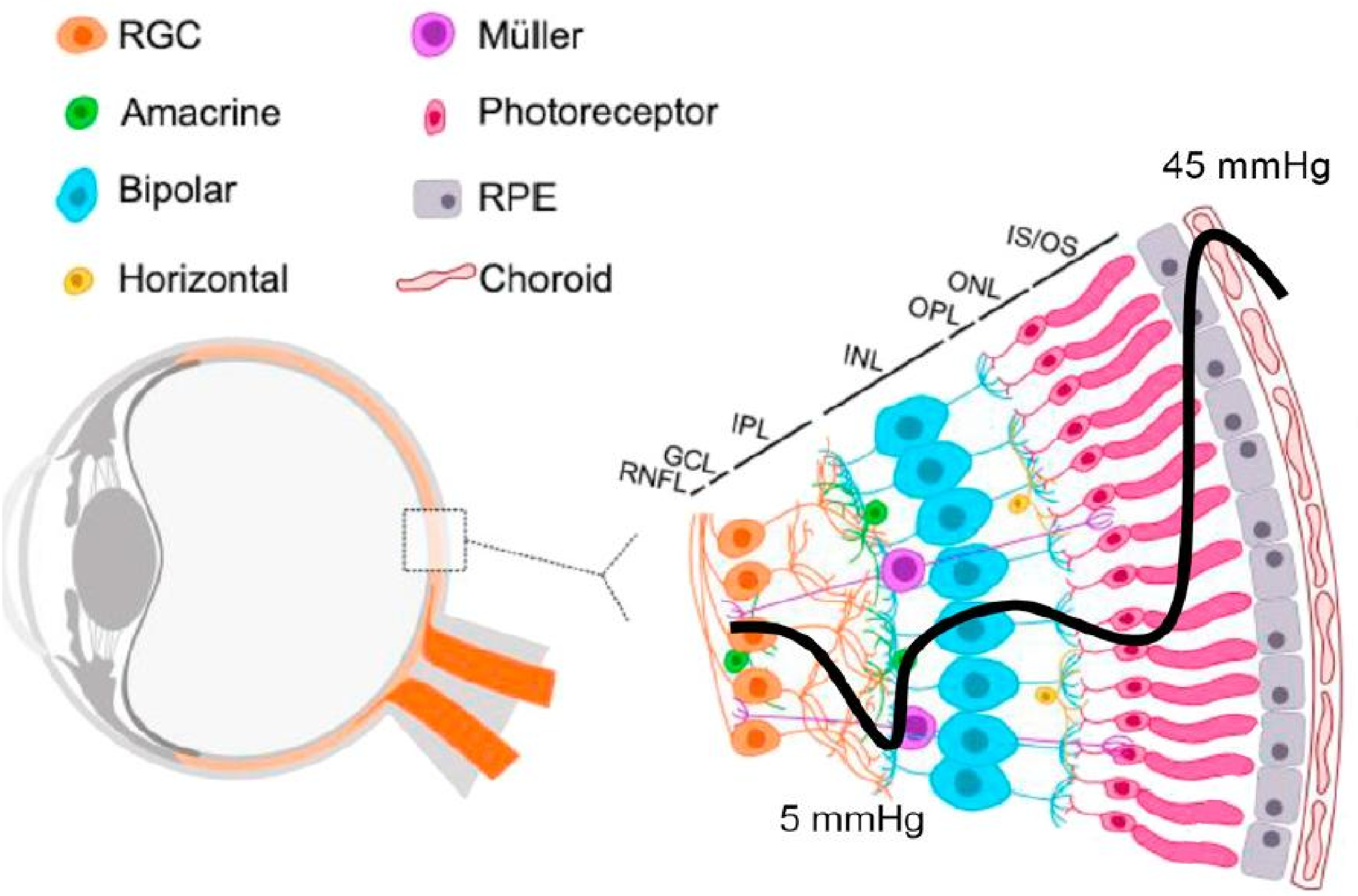




Agilent

Wide range of applications brings a new perspective of disease research

能量代谢——眼睛



- 晶状体主要通过无氧糖酵解途径获取能量
- 有氧情况下主要依赖糖酵解途径供能，称为视网膜Warburg效应

糖尿病视网膜病变

Science Translational Medicine   Current Issue   First release papers

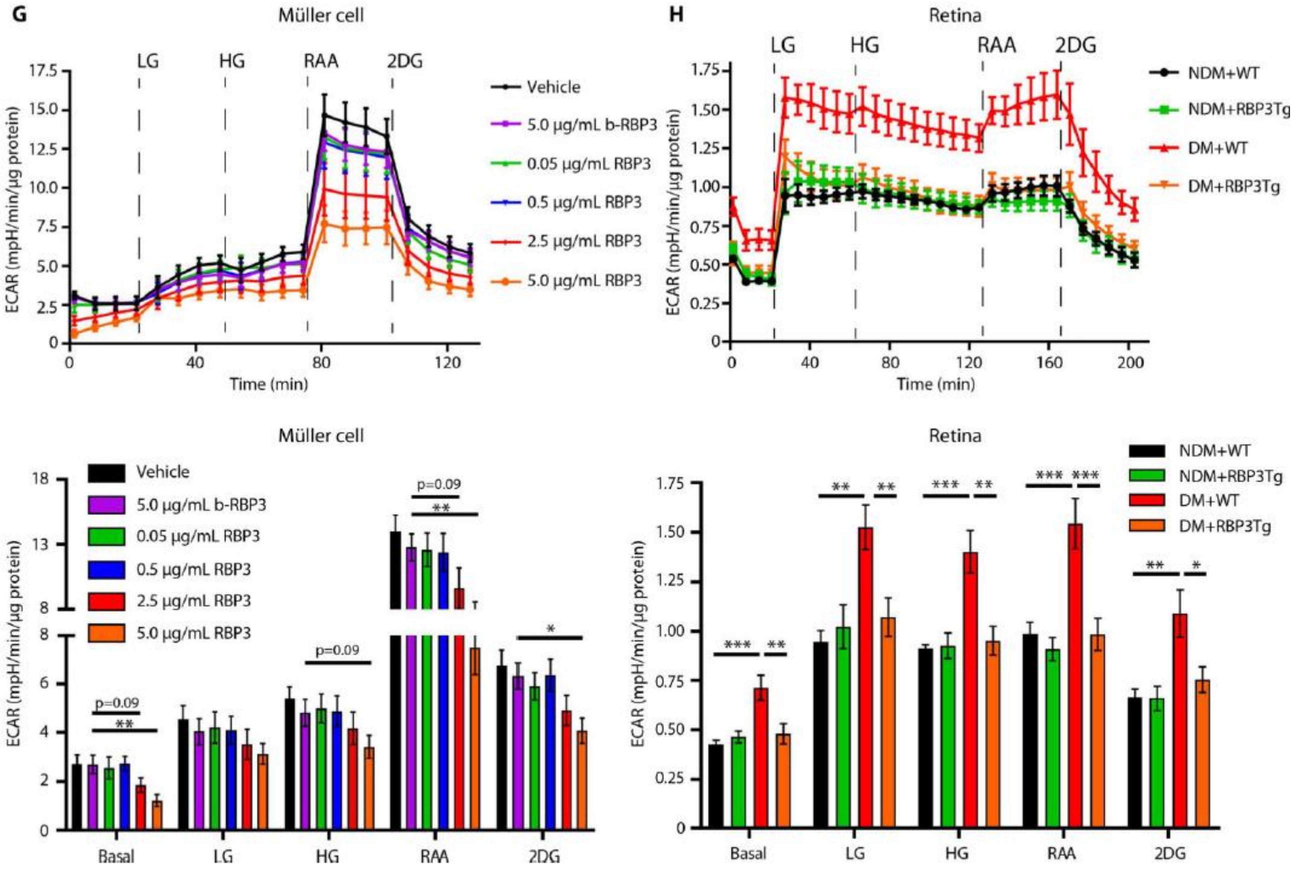
HOME > SCIENCE TRANSLATIONAL MEDICINE > VOL. 11, NO. 499 > RETINOL BINDING PROTEIN 3 IS INCREASED IN THE RETINA OF...

RESEARCH ARTICLE | DIABETIC RETINOPATHY

Retinol binding protein 3 is increased in the retina of patients with diabetes resistant to diabetic retinopathy

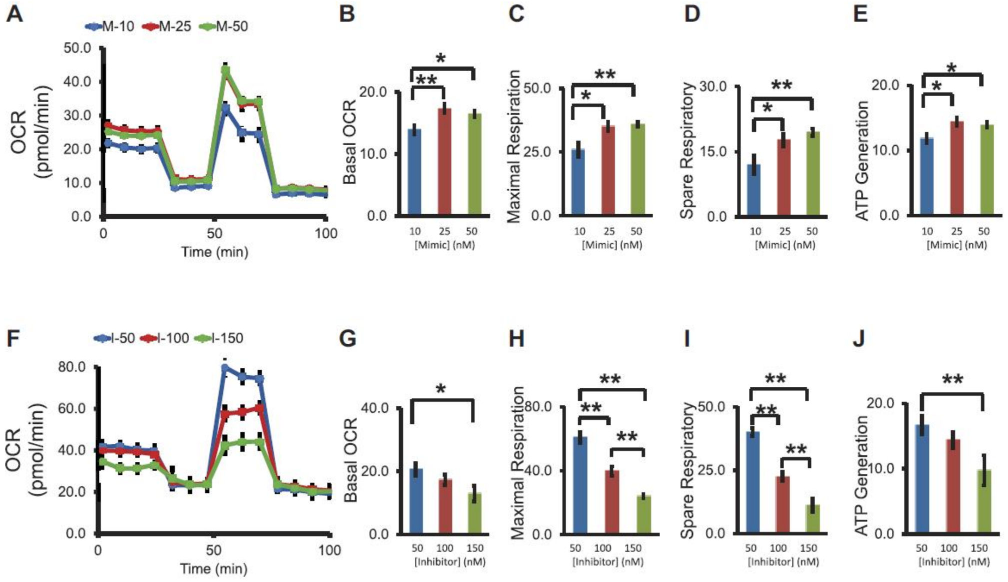
HISASHI YOKOMIZO, YASUTAKA MAEDA, KYOUNGMIN PARK, ALLEN C. CLERMONT, SONIA L. HERNANDEZ, WARD FICKWEILER, QIAN LI, CHIH-HAO WANG, SAMANTHA M. PANIAGUA, [...] GEORGE L. KING +14 authors Authors Info & Affiliations

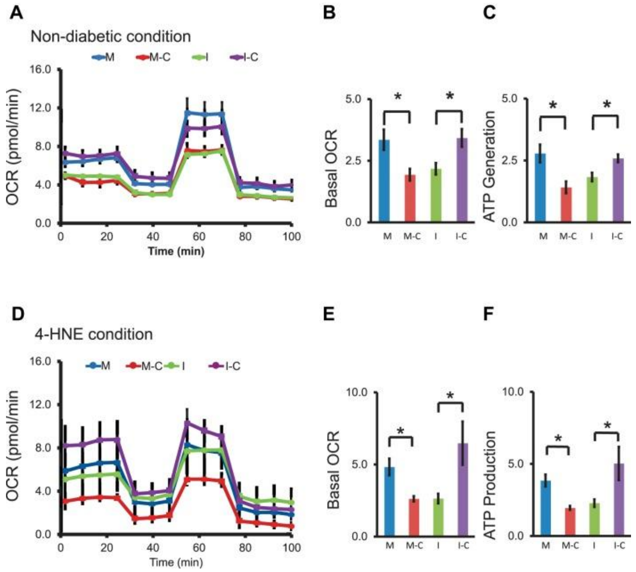
光感受器分泌的RBP3的高表达可过抑制GLUT1的葡萄糖摄取，降低炎性细胞因子和VEGF的表达，在防止高血糖引起的DR进展中发挥作用

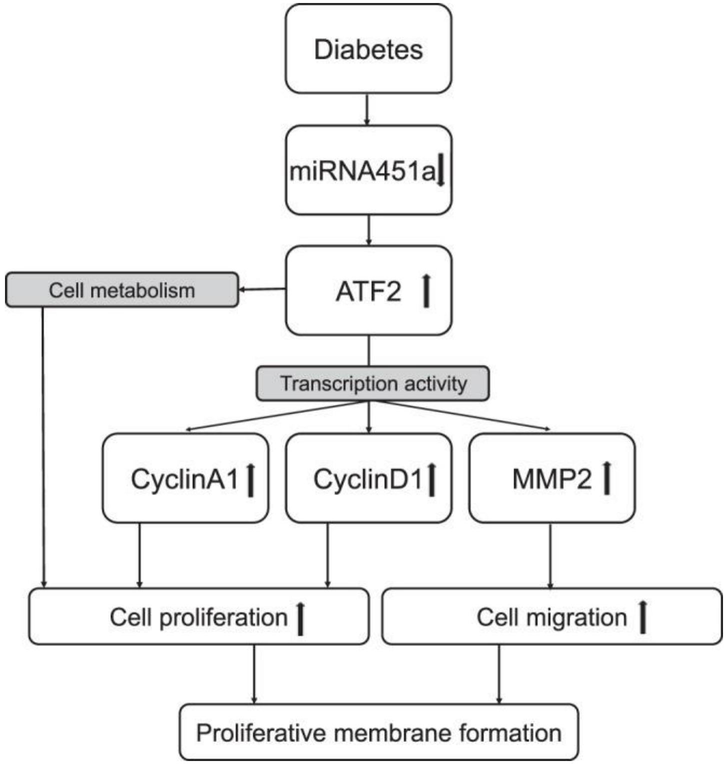


RESEARCH ARTICLE | *Mitochondria Dysfunction in Aging and Metabolic Diseases*

miRNA-451a regulates RPE function through promoting mitochondrial function in proliferative diabetic retinopathy







miR-451a对RPE的保护作用为PDR的治疗策略提供了新的治疗见解

Am J Physiol Endocrinol Metab 316: E443–E452, 2019.

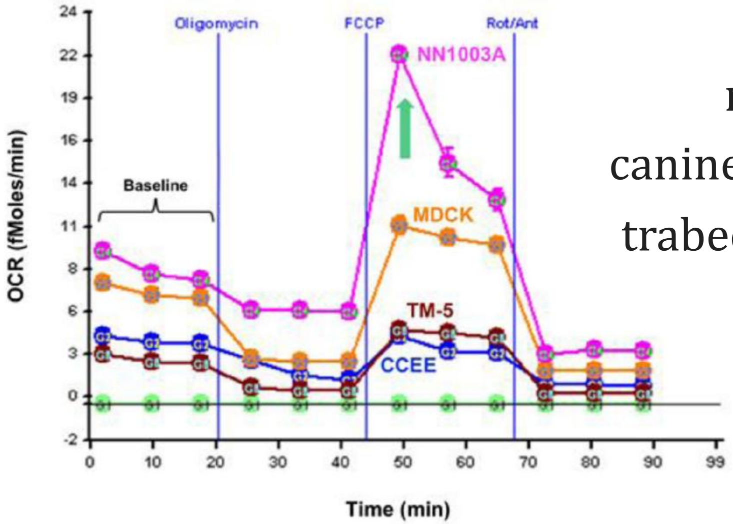
白内障

Free Radical Biology and Medicine
Volume 97, August 2016, Pages 513-519

Original article

Mitochondrial oxygen metabolism in primary human lens epithelial cells: Association with age, diabetes and glaucoma

M. Kubota [a, b], Y.B. Shui [a], M. Liu [a], F. Bai [a], A.J. Huang [a], N. Ma [a, d], D.C. Beebe [a, c], C.J. Siegfried [a]



rabbit LECs (NN1003A)
canine kidney epithelial cells (MDCK)
trabecular meshwork cells (TM-5)
CCEE

HIGHLIGHTS

- Measurement of oxygen consumption rate (OCR) in four mammalian cell lines
- Lens epithelial cells (LEC) exhibit higher OCR indicating specialized metabolism
- Mitochondrial function measurement of human LECs taken from surgical patients in vivo
- Older age, diabetes and glaucoma were associated with significantly lower OCR
- Decreased mitochondrial function in LECs may contribute to cataractogenesis

Cell Line	Median Age (passage)	Baseline OCR ± SD (fmol/min/cell)	Maximal OCR ± SD (fmol/min/cell)	O_2-dependent ATP Production (%)	Proton Leak (%)	Spare Resp. Capacity (fmol/min/cell)	Baseline ECAR ± SD (µpH/min/cell)
NN1003A	10	7.64 ± 0.37	21.60 ± 0.42	26%	74%	13.93	2.19 ± 0.49
MDCK	8	6.56 ± 0.19****	11.04 ± 0.60****	61%	39%	4.48	0.78 ± 0.28**
TM-5	7	2.54 ± 0.09****	4.57 ± 0.37****	67%	33%	2.03	0.61 ± 0.13**
CCEE	4	3.77 ± 0.09****	4.17 ± 0.22****	60%	40%	0.40	0.56 ± 0.14**

OCR = Oxygen Consumption Rate, ECAR = Extracellular Acidification Rate, Resp. = respiratory

**In relation to NN1003A, p<0.001

****In relation to NN1003A, p<0.0001

Age (years)		Gender		Race		Diagnosis			
						Diabetes		Glaucoma	
≤69	≥70	Female	Male	Caucasian	African American	No	Yes	No	Yes
36	33	47	22	44	25	48	21	35	34

Mitochondria Stress Test Results from Primary Culture Human Lens Epithelial Cells

Patient Characteristic	Baseline OCR (fmol/min/cell)	Maximal OCR (fmol/min/cell)	O2-dependent ATP Production (%)	Proton Leak (%)	Spare Resp. Capacity (fmol/min/cell)	Baseline ECAR (μpH/min/cell)
Age ≤69 years	**2.86***	5.28	32%	68%	2.37	2.04
Age ≥70 years	**2.21**	4.33	35%	65%	2.12	1.76
Non-Diabetic	**2.79***	5.20	33%	67%	2.41	1.93
Diabetic	**2.02**	3.96	34%	66%	1.94	1.86
No Glaucoma	**2.83***	**5.46***	33%	67%	2.59	2.13
Glaucoma	**2.27**	**4.17**	33%	67%	1.90	1.68
Female	2.63	4.85	32%	68%	2.19	2.09
Male	2.39	4.76	35%	65%	1.57	1.51
Caucasian	2.57	4.69	32%	68%	2.10	1.68
African American	2.53	5.05	36%	64%	2.53	2.31
Total (n=69)	2.55	4.82	33%	67%	2.27	1.91

*$p<0.05$

老年患者、青光眼患者和糖尿病患者晶状体上皮细胞（LEC）的线粒体功能下降，这些因素可能在白内障发生中起关键作用

Muller细胞

OPEN ACCESS

Retinal Cell Biology | March 2019

Dual Properties of Lactate in Müller Cells: The Effect of GPR81 Activation

Rupali Vohra; Blanca I. Aldana; Helle Waagepetersen; Linda H. Bergersen; Miriam Kolko

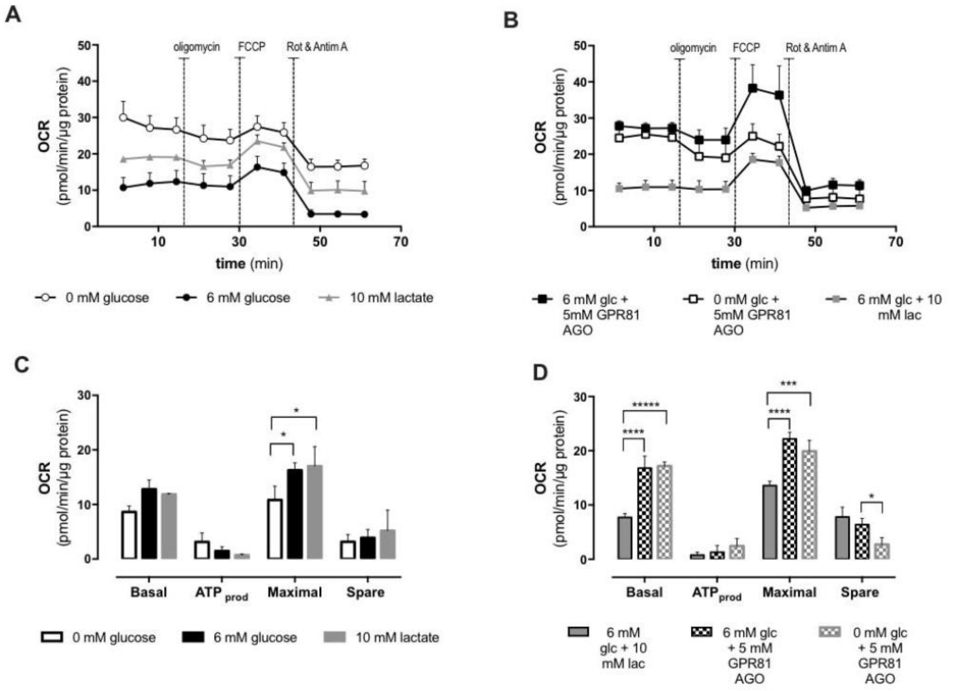
+ Author Affiliations & Notes

Investigative Ophthalmology & Visual Science March 2019, Vol.60, 999-1008.
doi:https://doi.org/10.1167/iovs.18-25458

Statistical comparison of mitochondrial respiration parameters

Compared conditions	parameter			
	Basal	**ATP production**	**Maximal**	**Spare**
GLC *vs.* GLC + GPR81 AGO	***0.01***	0.94	***0.02***	0.219
GLC *vs.* GLC + LAC	0.82	0.85	0.74	0.14
GLC + GPR81 AGO *vs.* 0 GLC + GPR81 AGO	0.85	0.55	0.285	0.07
0 GLC *vs.* 0 GLC + GPR81 AGO	***0.01***	0.83	***0.01***	0.88
GLC + LAC *vs* LAC	0.90	0.91	1.00	0.53
GLC + LAC *vs* GLC + GPR81 AGO	***0.0004***	0.76	**0.0003**	0.41
GLC + LAC *vs* 0 GLC + GPR81 AGO	***0.0003***	0.39	**0.004**	***0.02***

2-way ANOVA was used for statistical analyses, P<0.05 was considered significant



GPR81激活增加原代Müller细胞的线粒体活性

乳酸双重特性：
1.乳酸作为活性代谢能量底物
2.激活原代Müller细胞中GPR81受体的调节分子的新特性

A Protective Effect of PPARα in Endothelial Progenitor Cells Through Regulating Metabolism

Yan Shao,[1,2,3] Jianglei Chen,[3] Li-jie Dong,[1,3] Xuemin He,[3] Rui Cheng,[3] Kelu Zhou,[3] Juping Liu,[1] Fangfang Qiu,[3] Xiao-rong Li,[1,2] and Jian-xing Ma[3,4]

Diabetes 2019;68:2131–2142 | https://doi.org/10.2337/db18-1278

Retinal Cell Biology

The Mitochondrial-Derived Peptide Humanin Protects RPE Cells From Oxidative Stress, Senescence, and Mitochondrial Dysfunction

Parameswaran G. Sreekumar,[1] Keijiro Ishikawa,[1] Chris Spee,[2] Hemal H. Mehta,[3] Junxiang Wan,[3] Kelvin Yen,[3] Pinchas Cohen,[3] Ram Kannan,[1] and David R. Hinton[2,4]

LETTERS

nature medicine

Retinal lipid and glucose metabolism dictates angiogenesis through the lipid sensor Ffar1

Jean-Sébastien Joyal[1–3], Ye Sun[4], Marin L Gantner[5], Zhuo Shao[4], Lucy P Evans[4], Nicholas Saba[4], Thomas Fredrick[4], Samuel Burnim[4], Jin Sung Kim[3], Gauri Patel[2], Aimee M Juan[4], Christian G Hurst[4], Colman J Hatton[4], Zhenghao Cui[4], Kerry A Pierce[6], Patrick Bherer[7], Edith Aguilar[8], Michael B Powner[9], Kristis Vevis[9], Michel Boisvert[10], Zhongjie Fu[4], Emile Levy[10], Marcus Fruttiger[9], Alan Packard[11], Flavio A Rezende[12], Bruno Maranda[7], Przemyslaw Sapieha[12], Jing Chen[4], Martin Friedlander[8], Clary B Clish[6] & Lois E H Smith[4]

ORIGINAL ARTICLE

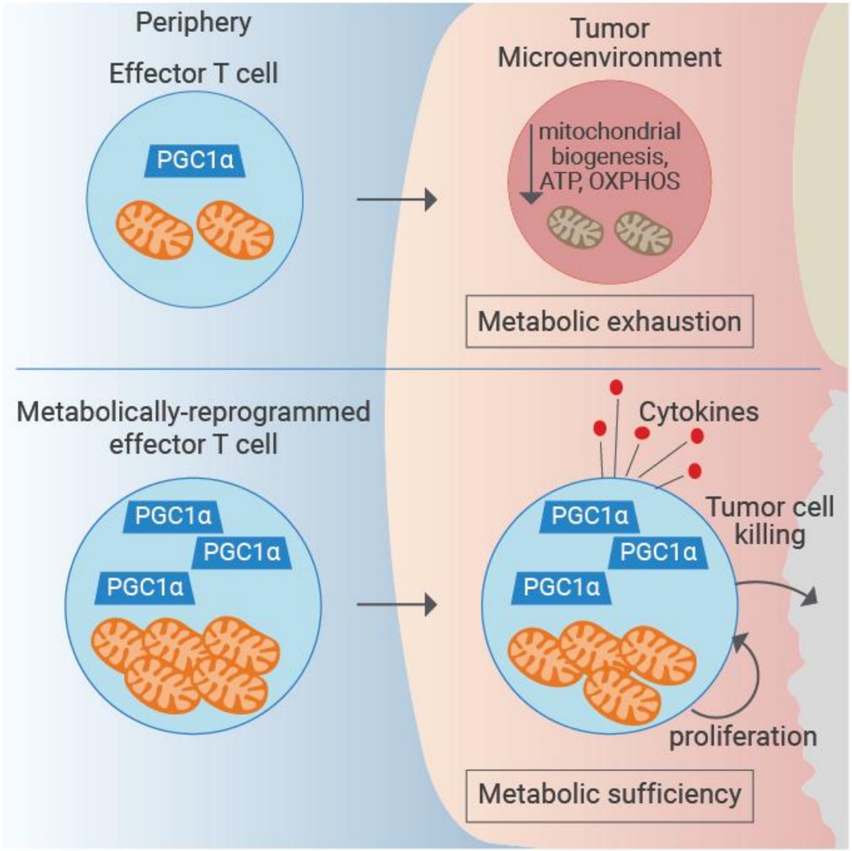
CLINICAL GENETICS WILEY

Whole-exome sequencing identified *ARL2* as a novel candidate gene for MRCS (microcornea, rod-cone dystrophy, cataract, and posterior staphyloma) syndrome

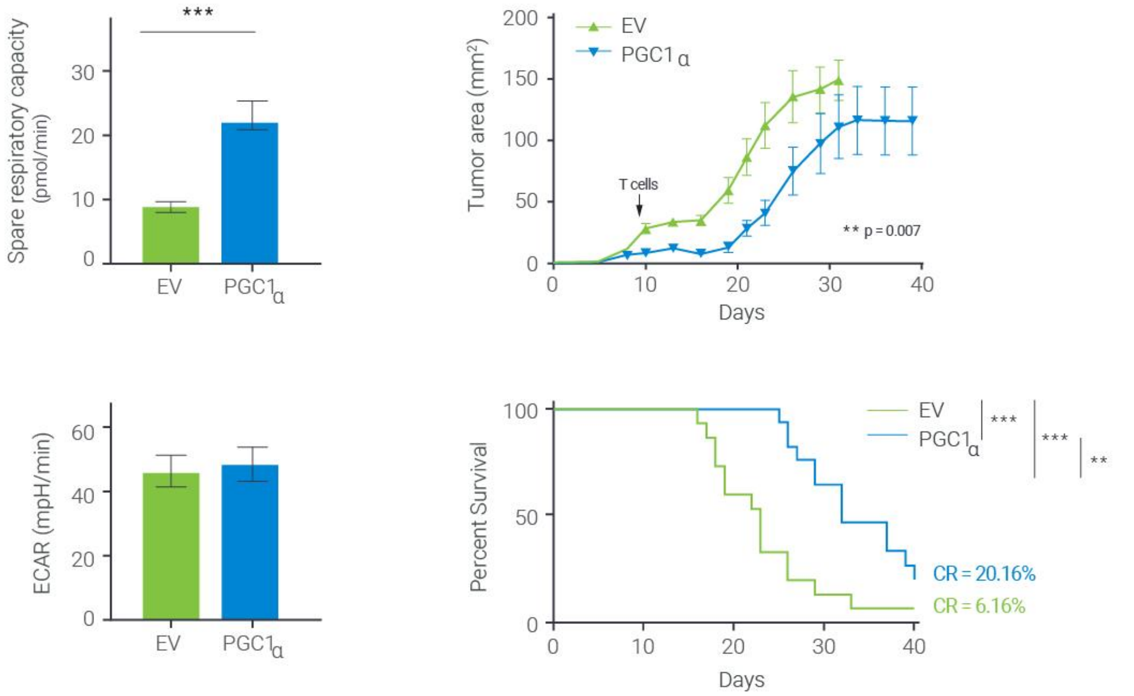
Xue-Bi Cai[1,3] | Kun-Chao Wu[1,3] | Xiao Zhang[1,3] | Ji-Neng Lv[1,3] | Guang-Hui Jin[1,3] | Lue Xiang[1,3] | Jie Chen[3] | Xiu-Feng Huang[1,3] | Deng Pan[1,3] | Bin Lu[2] | Fan Lu[3] | Jia Qu[3] | Zi-Bing Jin[1,3]

Metabolic reprogramming by genetic engineering of immune cells can restore anti-tumor function

TME represents a restrictive environment



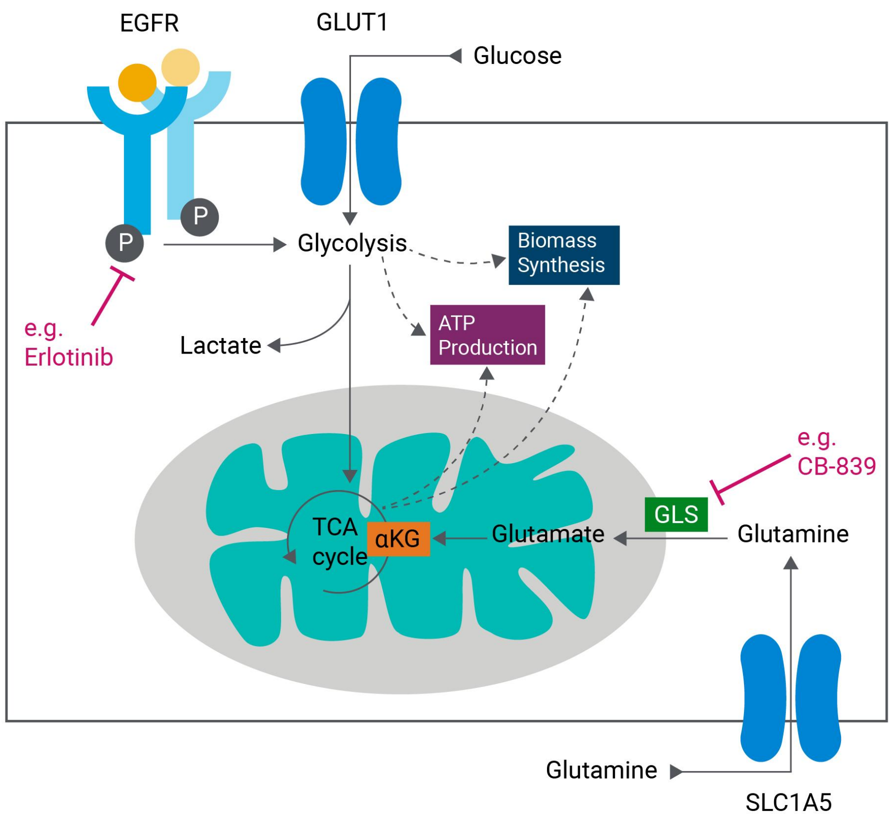
Enhanced mitochondrial biogenesis, through expression of PGC1-α, results in restoration of anti-tumor function in the TME

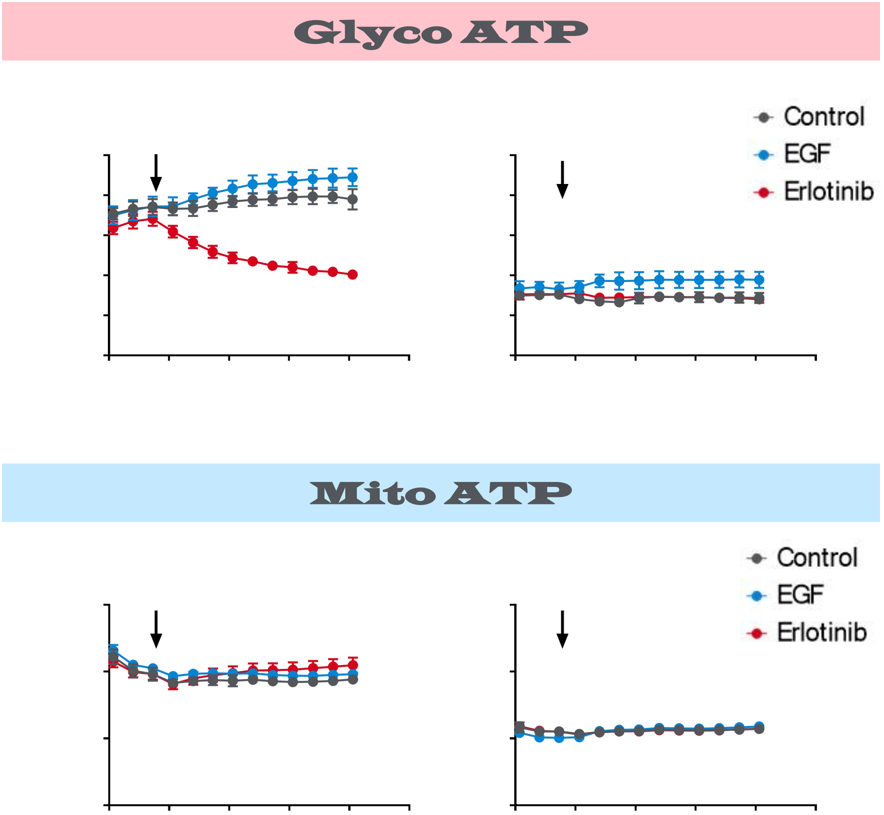


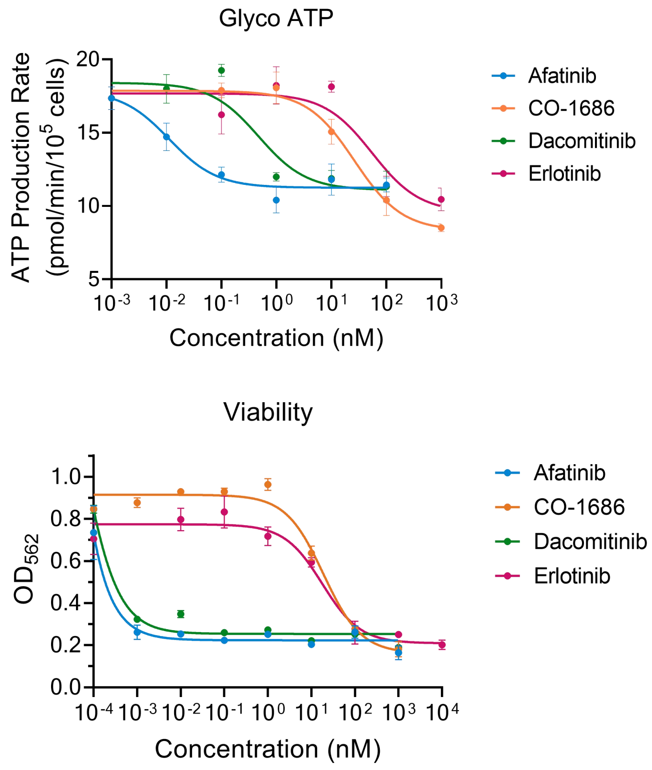
Adapted from Scharping et al., (2016), Immunity 45, 374–388

Metabolic Target Identification and Validation

Metabolic approach to EGFR-targeted therapy in non-small cell lung cancer







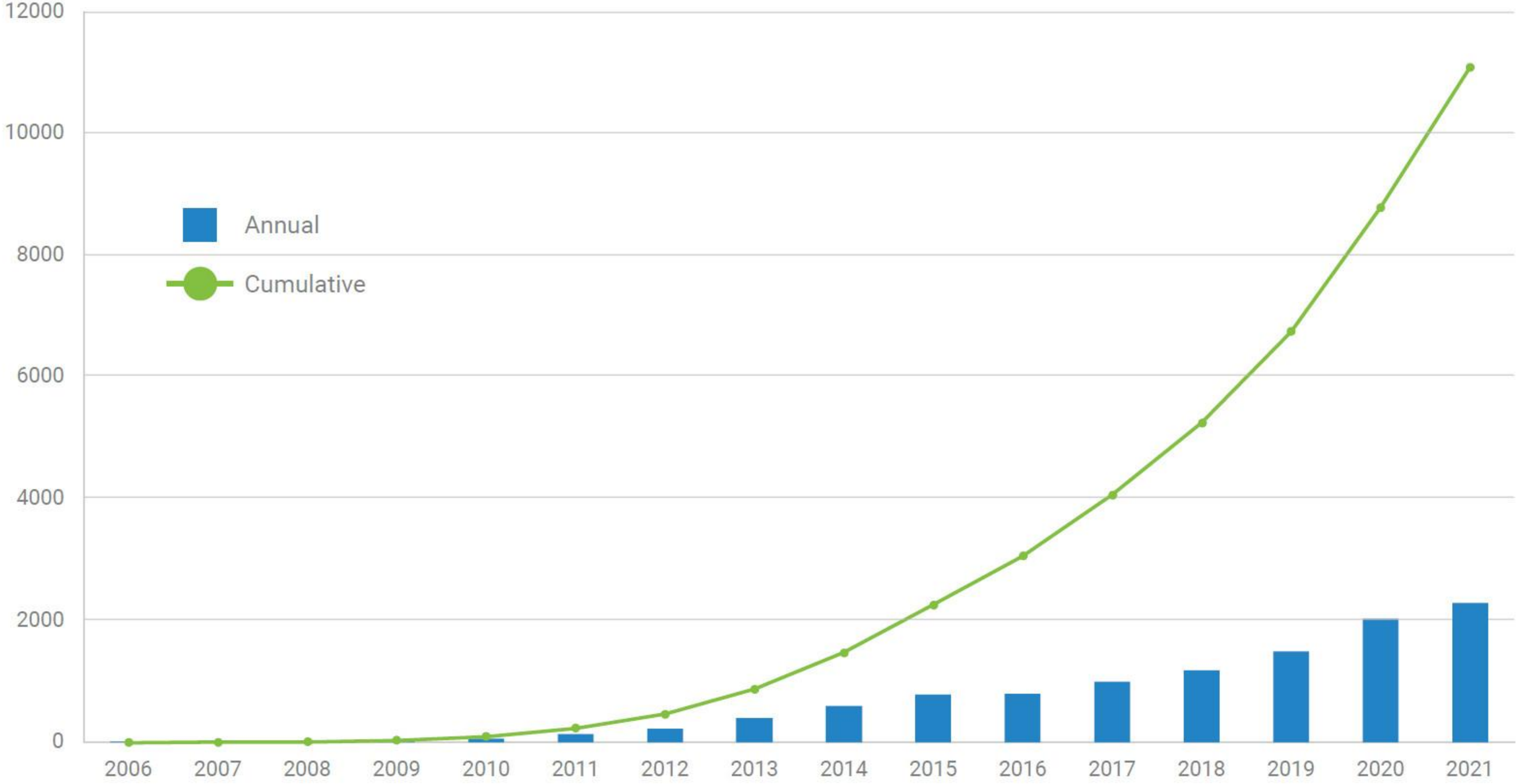
Adapted from Momcilovic et al. (2017) Cell Reports.

PC9: EGFR mutated NSCLC
A549: KRAS mutated NSCLC

Nature. 2023 Oct;622(7983):619-626

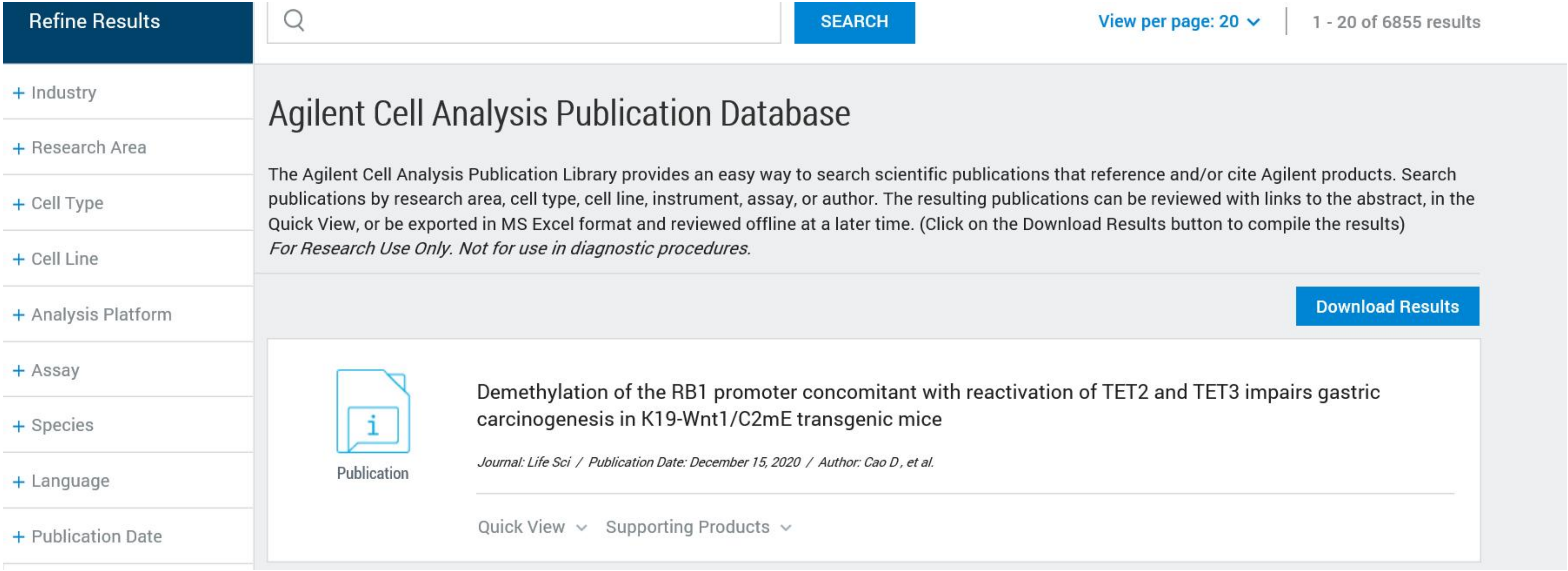
Nearly 11,000 publications to date

Agilent cell analysis publications (through 2021)



More Applications

https://www.agilent.com/search/?N=4294836537



Thank You!