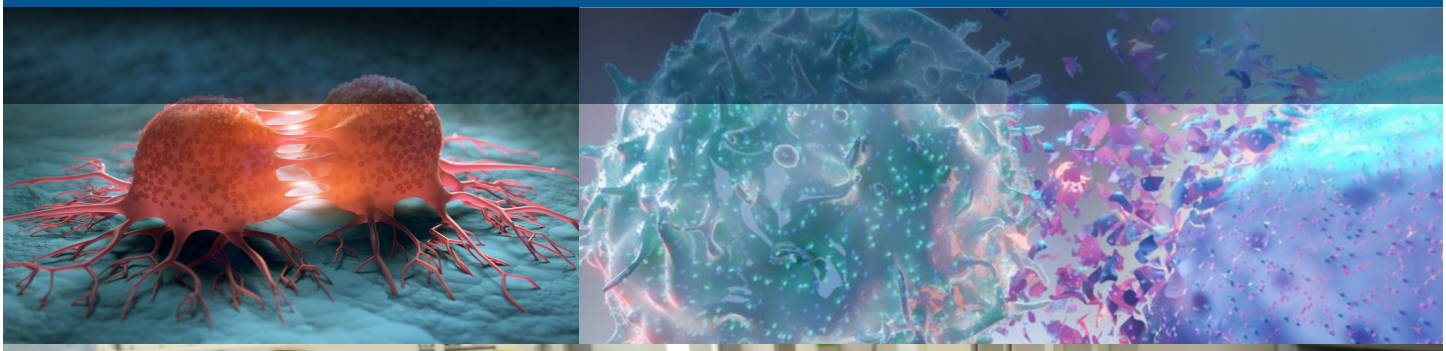
Agilent

Trusted Answers

Real-Time Metabolic Analysis for Cancer Research

Agilent Seahorse XF technology



Metabolic reprogramming is a hallmark of cancer, and a critical driver of all other hallmarks

Exploiting metabolic liabilities for therapeutic targeting

Cancer is a diverse collection of diseases linked to genetic changes that affect normal cell function, and metabolic reprogramming is emerging as a critical target in therapeutic intervention. Cancer cells are highly dependent on metabolic pathways to generate the necessary energy for many oncogenic processes, including rapid proliferation, survival, invasion, and metastasis, and will reprogram their metabolism to support these processes.

Today, researchers use metabolic analysis tools together with other cell-based assays to further their understanding of cancer biology. Investigating the dynamic nature of cellular metabolism and how cancer cells reprogram their metabolism to adapt and survive using functional measurements in real time can reveal metabolic liabilities. These metabolic liabilities can then be exploited for therapeutic targeting.

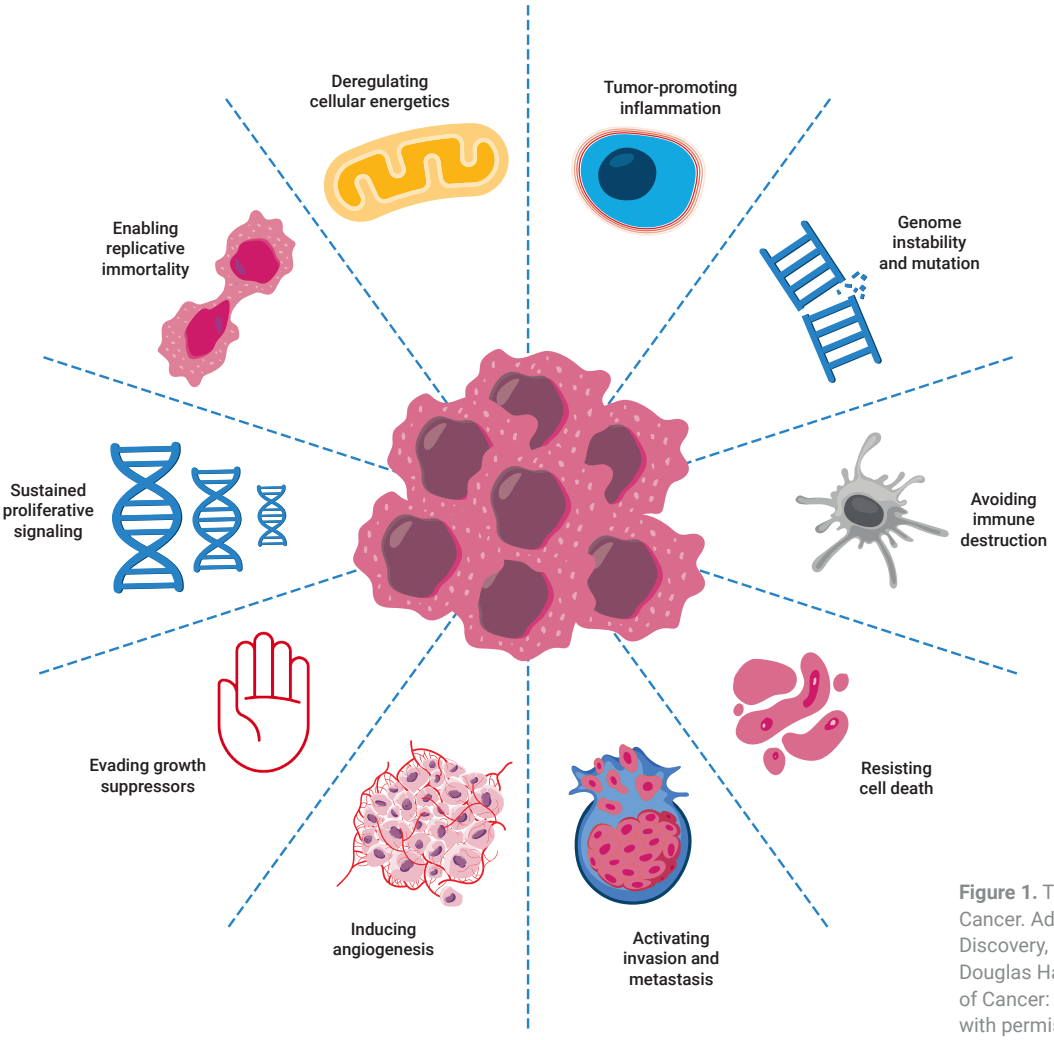


Figure 1. The Hallmarks of Cancer. Adapted from Cancer Discovery, 2022, 12(1), 31-46, Douglas Hanahan, Hallmarks of Cancer: New Dimensions, with permission from AACR.

Seahorse XF solutions for cancer research

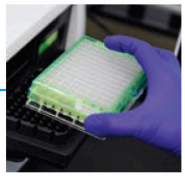
Generate functional measurements in real time

The Agilent Seahorse XF platform provides functional measurements of two primary metabolic pathways—glycolysis and oxidative phosphorylation—from live cells in real time. This technology enables the phenotypic evaluation of cancer cells in response to different metabolic substrates or inhibitors.

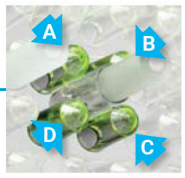
State-of-the-art
data analytics tools

Real-time
calculation of results

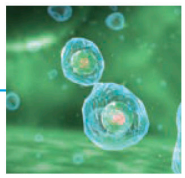
Validated
kits, media, and reagents



Label-free
pH and O_2 sensor cartridge



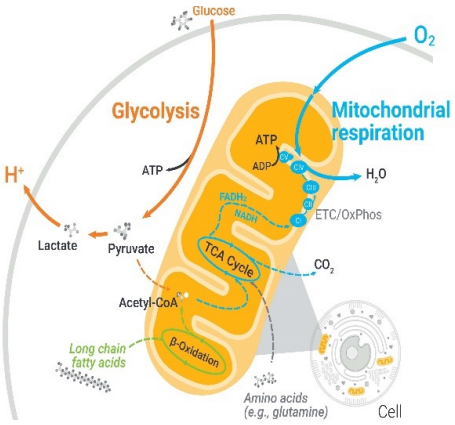
More relevant
injection ports for real-time modulation



Live-cell analysis
with 2D and 3D plate options

Discover why cancer researchers are using Seahorse XF cell analysis technology to investigate:

- Metabolic phenotyping for disease models
- Cancer substrate dependencies, plasticity, and vulnerabilities in the tumor microenvironment (TME)
- Signaling or pathway intermediates, target identification/validation, mechanism of action, and checkpoint blockade
- Immuno-oncology and immune cell fitness versus exhaustion



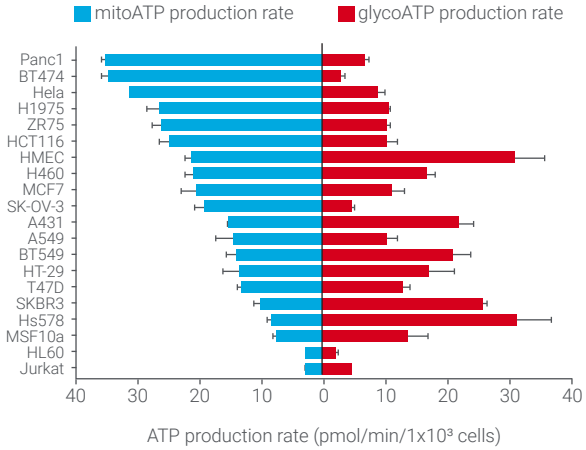
Find out more about the Agilent Seahorse XF Pro analyzer, **click here**.

Cancer cell dependencies and adaptation strategies go beyond glycolysis

Define the variabilities in metabolic phenotypes driving cancer vulnerabilities

Cancer is a metabolic disease, which is often characterized by a Warburg effect with upregulated glycolysis. However, metabolic phenotypes are substantially variable, and can serve as a critical predictor of cancer proliferation, vulnerabilities, and resistance to therapies. Cell analysis with Seahorse XF technology can provide a direct measure of functional live cell metabolism, illuminating the cancer vulnerabilities that drive cancer cell progression and proliferation.

Cancer metabolic phenotypes and vulnerabilities are highly diverse



Cancer cells have developed different strategies for cellular energy production, with significant implications for therapeutic strategy. Measuring ATP production rates across a panel of 20 cancer cell lines with the Agilent Seahorse XF Real-Time ATP rate assay reveals a wide range of energy phenotypes, from predominantly oxidative (Figure 2, top) to predominantly glycolytic (Figure 2, bottom).

Figure 2: From Romero et al. Bioenergetic profiling of cancer cell lines: quantifying the impact of glycolysis on cell proliferation, Agilent Technologies Poster, AACR, **2018**.

Invasive estrogen-receptor-positive breast cancer cells are characterized by a more oxidative metabolic phenotype

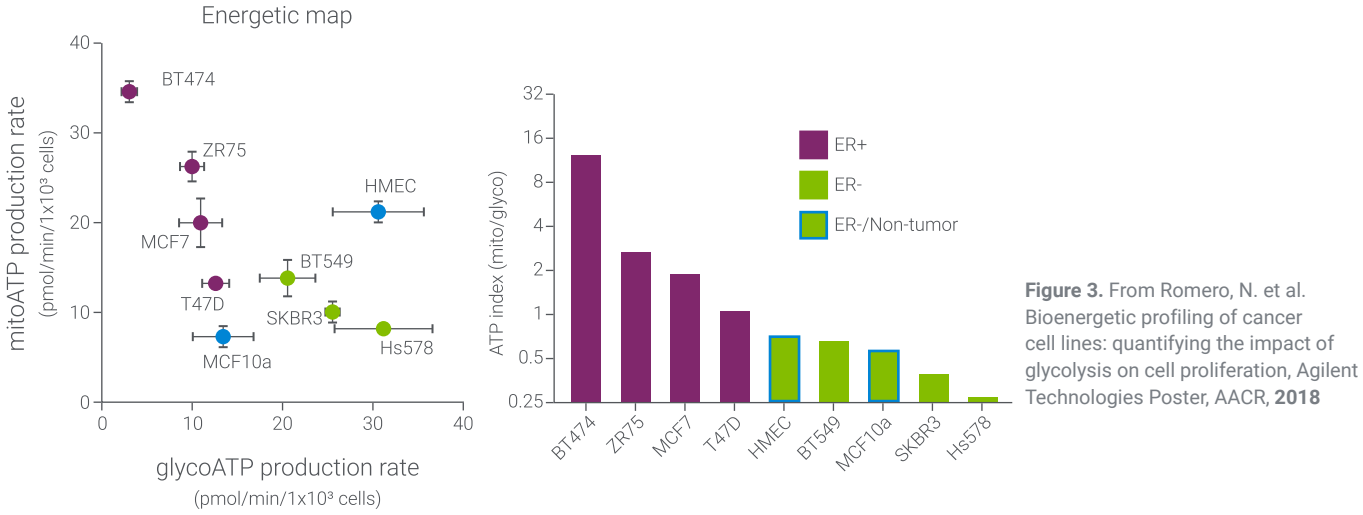


Figure 3. From Romero, N. et al. Bioenergetic profiling of cancer cell lines: quantifying the impact of glycolysis on cell proliferation, Agilent Technologies Poster, AACR, **2018**

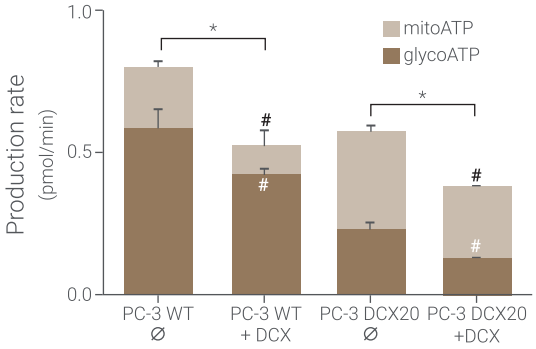
The energetic map in Figure 3 shows the distribution of mitoATP production rate versus glycoATP production rate across seven cancer cell lines and two normal breast-derived cell lines. Analysis of the metabolic index (mitoATP production rate / glycoATP production rate) shows that estrogen-receptor-positive (ER+) breast cancer cell lines have a higher metabolic index.

Measure dynamic changes in cancer cell metabolism

Rapid changes in metabolism are a critical strategy in chemoresistance

Cancer proliferation is a rapid and dynamic process that demands significant biochemical energy. As a result, cancer cells exhibit an altered metabolism that may rely on one or both of the main metabolic pathways—glycolysis or oxidative phosphorylation. The ability of some cancer cells to switch between pathways is a key strategy driving cancer cell adaptation. Seahorse XF technology enables simultaneous measurements of the two major metabolic pathways in live cells in real time.

Cancer cells rapidly exploit metabolism to adapt and survive through metabolic plasticity



Prostate cancer cells (PC-3) predominantly use glycolysis for ATP generation. However, Catapano et al, showed that the drug-resistant lineage, PC-3_DCX20, established from long-term docetaxel (DCX) treatment, displays a higher reliance on oxidative phosphorylation for ATP generation (Figure 4), demonstrating metabolic plasticity.

Figure 4. Adapted from Catapano, J., et al. (2022) Acquired drug resistance interferes with the susceptibility of prostate cancer cells to metabolic stress. Cell Mol Biol Lett, 27(1), 100, under the creative commons license 4.0 http://creativecommons.org/licenses/by/4.0/.

Seahorse XF technology reveals potential therapeutic targets for chemotherapy-resistant cancers

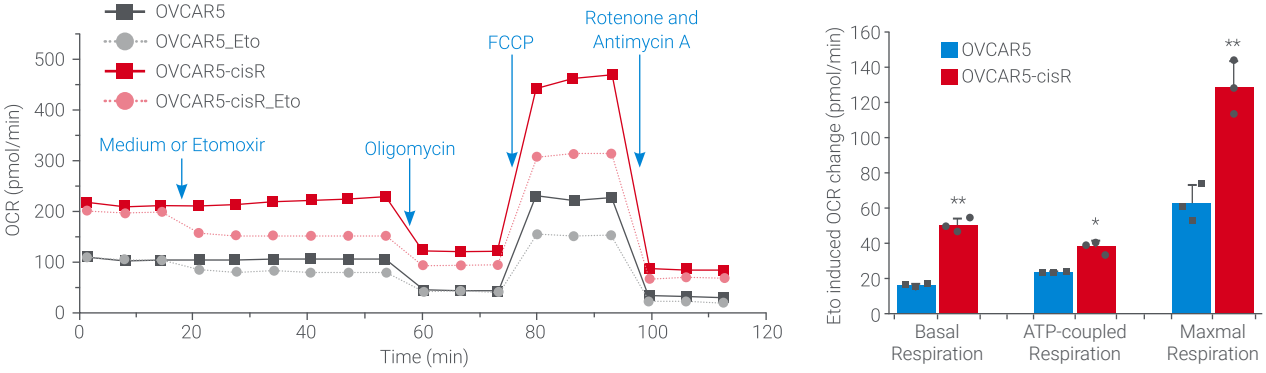


Figure 5. Adapted from Tan, Y., et al. (2022) Metabolic reprogramming from glycolysis to fatty acid uptake and beta-oxidation in platinum-resistant cancer cells. Nat Commun, 13 (4554), under the creative commons license 4.0 http://creativecommons.org/licenses/by/4.0/.

Tan and colleagues (Figure 5) identified a stable metabolic switch towards fatty acid oxidation (FAO)-dependent energy metabolism in cisplatin-resistant ovarian cancer cells. Seahorse XF assays were used to show that FAO is significantly increased in cisplatin-resistant ovarian cancer cells compared to their parental counterparts. Their study points towards targeting the FAO pathway as a potential therapeutic strategy for cisplatin-resistant cancers.

Exploit substrate dependencies of cancer cells with combination therapy

Cancer cells may alter lipid or amino acid metabolism or shift the balance between anabolic and catabolic processes to adapt to the nutritional conditions of the tumor microenvironment (TME). These processes may be analyzed directly via metabolic measurements.

Discover how Agilent cell analysis technology and metabolic phenotyping can provide insights into:

- Cellular dependencies, including fuels and microenvironment
- Metabolic vulnerabilities to inform druggable target identification
- Cancer drug development and efficacy

Metabolic vulnerabilities can reveal therapeutic targets for overcoming chemoresistance

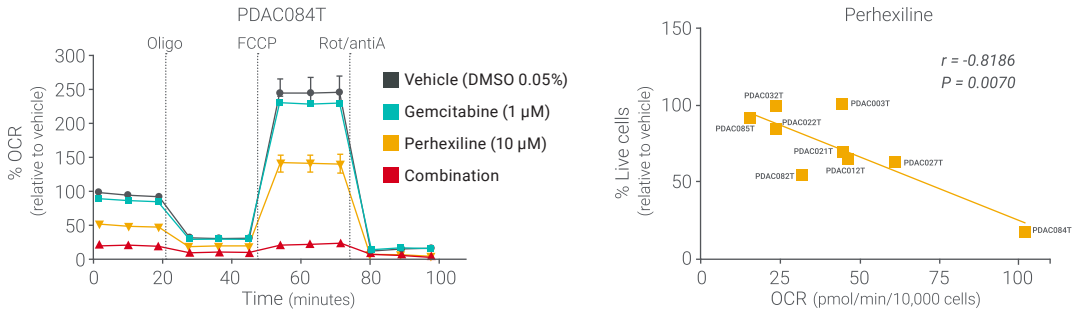


Figure 6. Adapted from Reyes-Castellanos, G. et al. (2023) Combining the anti-anginal drug perhexiline with chemotherapy induces complete pancreatic cancer regression in vivo. iScience, 26, 106899.

Reyes-Castellanos and colleagues (Figure 6) used Seahorse XF assays to show that mitochondrial respiration in pancreatic ductal adenocarcinoma (PDAC) cells depends mainly on FAO, revealing a potential metabolic vulnerability for pancreatic cancer. They showed that basal oxygen consumption rate (OCR) served as a biomarker for PDAC cell response to perhexiline—an FAO inhibitor. Combining perhexiline treatment with the chemotherapy gemcitabine enhanced led to an energetic crisis in PDAC cells and induced complete pancreatic cancer regression in one PDAC xenograft.

Agilent Seahorse XF technology differentiates the mechanisms of two lactate uptake inhibitors and antitumor drugs in whole cells and isolated mitochondria

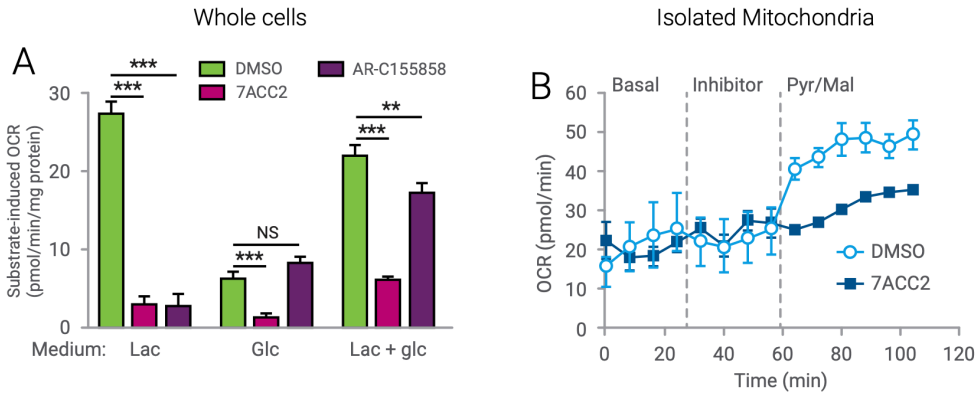


Figure 7A, 7B: Adapted from Corbet, C., et al. (2018) Interruption of lactate uptake by inhibiting mitochondrial pyruvate transport unravels direct antitumor and radiosensitizing effects. Nat Commun, 9 (1): 1208, under the creative commons license 4.0 http://creativecommons.org/licenses/by/4.0/

The Seahorse XF analyzer first determined that, unlike lactate inhibitor AR-C155858, the compound 7ACC2 fulfills the tasks of blocking lactate use while preventing oxidative metabolism of glucose (Figure 7A, whole cervical cancer cells). Using isolated mitochondria, the Seahorse XF analyzer further reveals that 7AAC2 works to inhibit lactate uptake via inhibition of the mitochondrial pyruvate carrier, which is a novel mechanism (Figure 7B, isolated mitochondria).

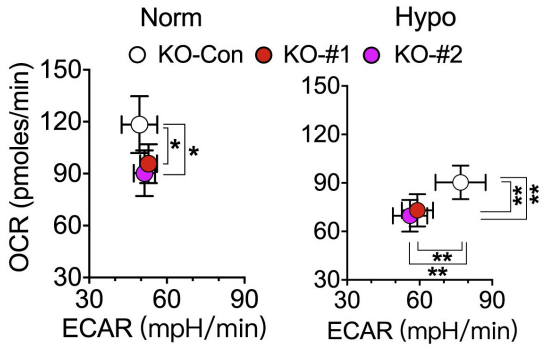
Discover how researchers are modeling the tumor microenvironment

The tumor microenviroment (TME) is a uniquely hypoxic and acidic environment that can promote cancer progression. By considering as many elements of the TME as possible, researchers have the greatest opportunity to produce effective treatments.

Seahorse XF technology can be used to model the TME:

- Agilent Seahorse XFe24 and XF Pro analyzers are compatible with hypoxia studies
- The Seahorse XF Pro analyzer provides a 3D spheroid microplate option

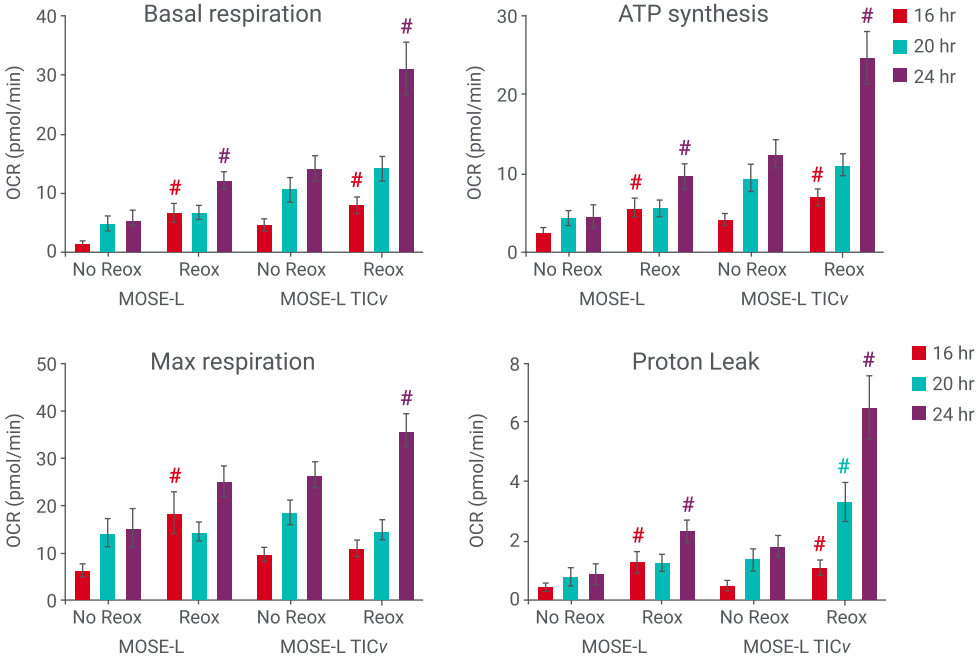
Generate insights into how cancer cells adapt their metabolism to hypoxia



Seahorse XF technology was used in a publication by Yang and colleagues (Figure 8), to generate insights into the role of mitochondrial UQCC3 in the adaptation of hepatocellular carcinoma (HCC) cells to hypoxia. They showed that UQCC3 forms a positive feedback loop with reactive oxygen species in hypoxic HCC cells, which maintains mitochondrial structure and function and stabilizes HIF-1α expression enhancing glycolysis.

Figure 8. From Yang, Y., et al. (2020) Mitochondrial UQCC3 Modulates Hypoxia Adaptation by Orchestrating OXPHOS and Glycolysis in Hepatocellular Carcinoma. Cell Reports, 33 (5), 108340.

Analyse metabolic adaptations of tumor spheroids to changes in culture conditions

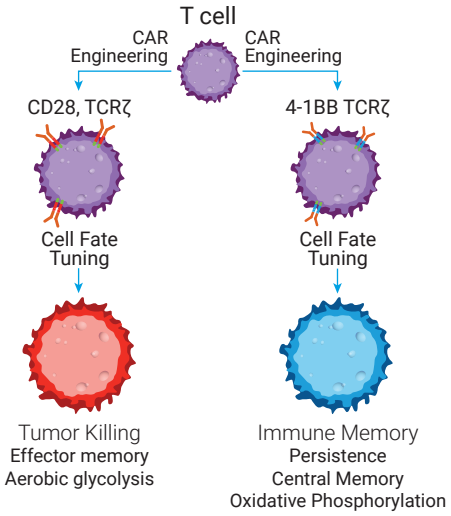


Greico and colleagues (Figure 9) used Seahorse XF assays during their studies into mitochondrial plasticity in ovarian cancer spheroids upon adhesion. They showed that adhesion reversed mitochondrial fragmentation and significantly increased OCR in both slow-growing MOSE-L spheroids and the more aggressive MOSE-LTICv spheroids, especially after re-oxygenation.

Figure 9. From Grieco, J.P., et al. (2023) Mitochondrial plasticity supports proliferative outgrowth and invasion of ovarian cancer spheroids during adhesion. Front. Oncol.,12:1043670, under the creative commons license 4.0 http://creativecommons.org/licenses/by/4.0/.

Advance new therapeutic opportunities in immuno-oncology with metabolism

Discover strategies to perturbate pathways and control immune cell response to advance cell therapy developments



The goal of immune-cell-based therapies is to enhance the performance of native immune cells by expanding or modifying immune cells to alter relevant signaling pathways in a way that changes the cellular function. Seahorse XF technology provides critical measurements of live cells in real time, revealing the functional outcome of modulation strategies. Discover how modulation of immune cell responses via signaling, checkpoint blockade, or pathway perturbation is "functionalized" through changes in metabolic programming.

CAR construct design can enhance immune cell fitness through modulation of metabolism

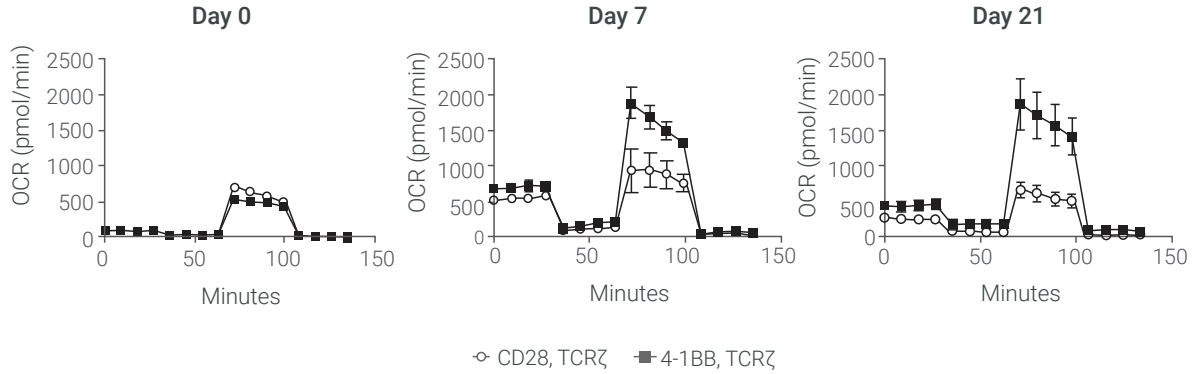


Figure 10. Adapted from Kawalekar, O., et al. (2016) Distinct Signaling of Coreceptors Regulates Specific Metabolism Pathways and Impacts Memory Development in CAR T Cells. Immunity, 44(2), 380–90.

Kawalekar et al. used the Seahorse XF assays to show that the choice of CAR signaling domain determines the bioenergetic phenotype of CD8+ CAR T cells postantigen stimulation. Over a 21-day period, CAR T cells containing the 4-1BB signaling costimulatory domain progressed towards exhibiting a greater SRC, which culminated in enhanced in vitro persistence and increased central memory differentiation relative to CAR T cells containing the CD28-signaling costimulatory domain.

Monitor metabolic changes in tumor cells to better characterize the tumor microenvironment and exploit checkpoint therapies

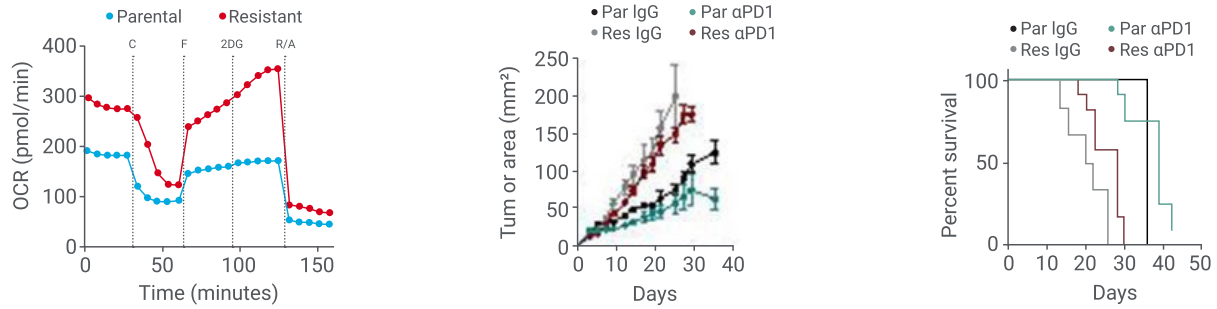


Figure 11. Adapted from Zandberg, D. et al. Tumor hypoxia is associated with resistance to PD-1 blockade in squamous cell carcinoma of the head and neck. J ImmunoTher Cancer 2021, 9(5), e002088.

Seahorse XF assays were used in studies by Zandberg and colleagues (Figure 11) to show that oxidative metabolism is upregulated as tumors become resistant to anti-PD-1 blockade. They demonstrated that the metabolic status of the TME can be predictive of tumor response to anti-PD-1 therapy.

Evaluate the impact of metabolic fitness on immune cell function

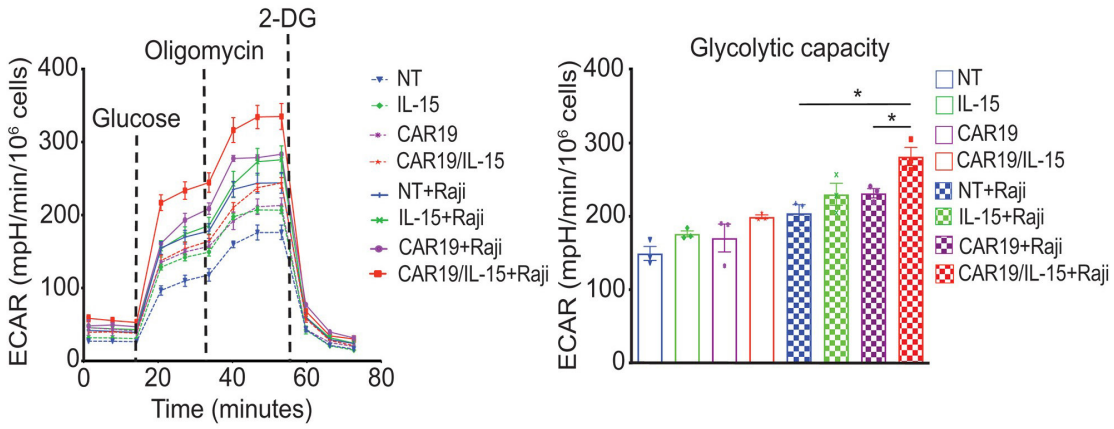


Figure 12. Adapted from Li, L. et al. Loss of metabolic fitness drives tumor resistance after CAR-NK cell therapy and can be overcome by cytokine engineering. Science Advances 2023, 9, eadd6997

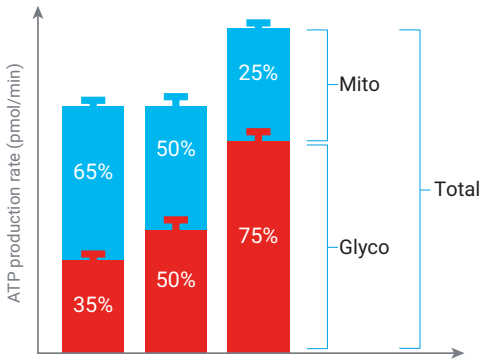
Li and colleagues (Figure 12) used Seahorse XF assays to show that engineering CAR19 NK cells to express interleukin 15 (IL-15) results in enhanced metabolic fitness with improved glycolytic activity compared to controls. Their study showed that the antitumor effects of CAR NK cells can be improved by increasing their metabolic fitness.

The Agilent Seahorse XF T Cell Metabolic Profiling kit is recommended for robust and accurate measurements of both glycolytic and mitochondrial activities in T and NK cell populations. Find out more **here**.

Seahorse XF assays for measuring cancer metabolism

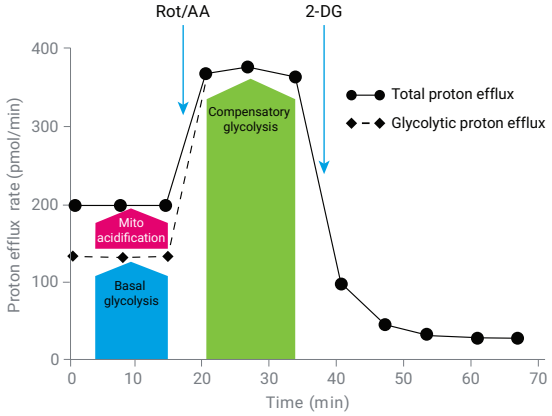
Agilent Seahorse XF Real-Time ATP rate assay kit

- For Agilent Seahorse XF Pro and XFe analyzers: part number 103592-100
- For Agilent Seahorse XF HS Mini and XFp analyzers: part number 103591-100



Agilent Seahorse XF Glycolytic rate assay kit

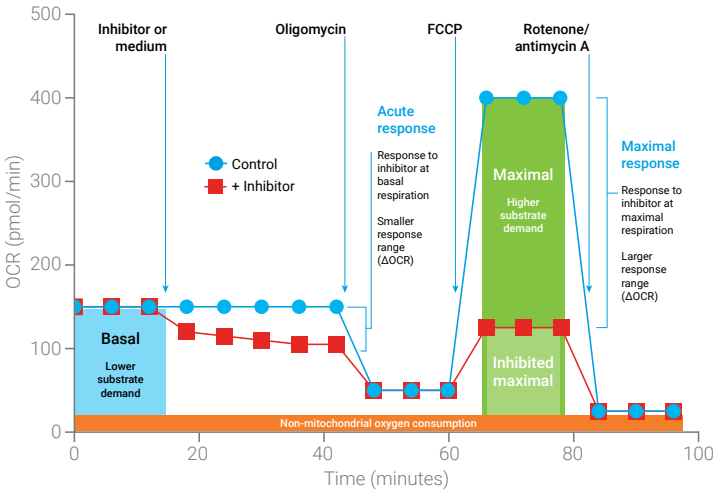
- For Seahorse XF Pro and XFe analyzers: part number 103344-100
- For Seahorse XF HS mini/XFp analyzers: part number 103346-100



Discover cancer vulnerabilities, plasticity, and metabolic phenotyping with simultaneous measurements of oxidative phosphorylation and glycolysis for comprehensive information about drivers of cell function. This information is now quantitative with the Seahorse XF Glycolytic and Real-Time ATP rate assay kits.

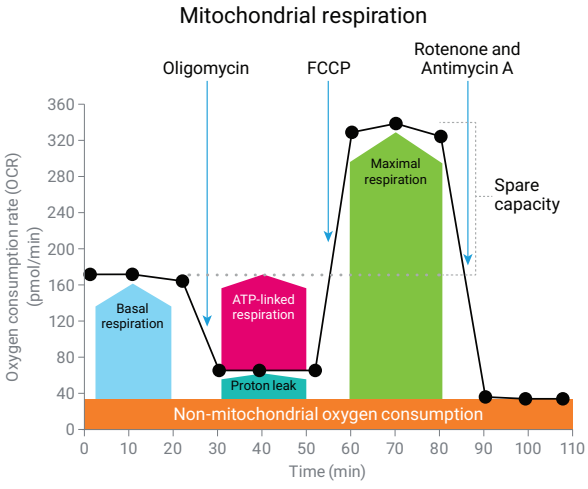
Agilent Seahorse XF Substrate Oxidation Stress Test kits

- Seahorse XF Long Chain Fatty Acid Oxidation Stress Test kit: part number 103672-100
- Seahorse XF Glucose/Pyruvate Oxidation Stress Test kit: part number 103673-100
- Seahorse XF Glutamine Oxidation Stress Test kit: part number 103674-1



Agilent Seahorse XF Mito Stress Test kit

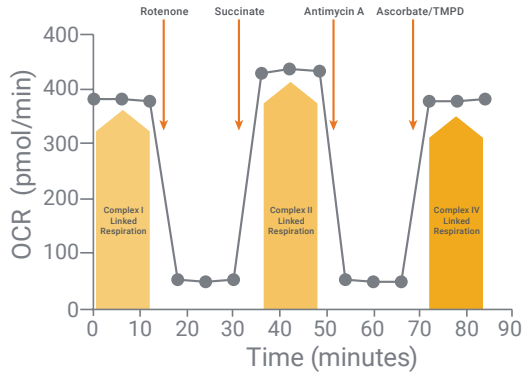
- For Seahorse XF Pro and XFe analyzers: part number 103015-100
- For Seahorse XF HS Mini and XFp analyzers: part number 103010-100



Investigate how cancer cells alter or shift oxidation of mitochondrial substrates to enhance proliferation, survive in the TME, or respond to genetic or pharmaceutical interventions.

Agilent Seahorse XF Plasma Membrane Permeabilizer

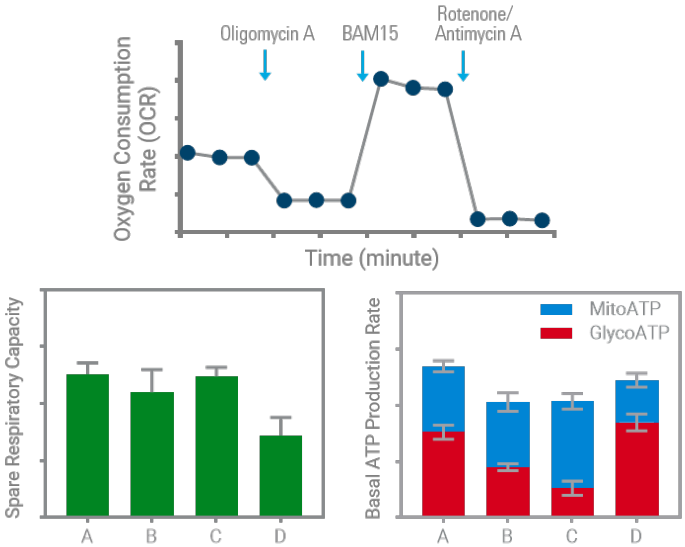
– Part number: 102504-100



Perform the same assays you would perform on isolated mitochondria, without isolating mitochondria. The exclusive reagent permeabilizes the plasma membrane of intact cells in culture without damage to mitochondrial membranes. This enables experimental control of substrate provision to the mitochondria and detailed characterization of key components in mitochondrial function, such as transporters, enzymes, and electron transport chain complexes.

Agilent Seahorse XF T Cell Metabolic Profiling kit: Customized assays for cell therapeutics development

With optimized reagents for different T cell and NK cell populations, these assays provide robust bioenergetic parameters linked to critical attributes for antitumor properties: cell persistence and metabolic fitness.



– Suitable for evaluation of construct design, engineering strategies, starting material selection, or metabolic conditioning during in vitro cell expansion
– Applicable for use in assessing the capacity of T cells and NK to maintain metabolic fitness in TMEs
– Includes BAM15—an improved uncoupler for more consistent and accurate measurements of T cell and NK cell mitochondrial function
– Provides a comprehensive view of T cell and NK cell metabolism, including simultaneous quantification of both glycolytic and mitochondrial effects, activity, and bioenergetic capacity
– Validated for both T cell and NK cell metabolic profiling

Explore Agilent Seahorse XF assay kits **here**.

U.S. and Canada
1-800-227-9770
agilent_inquiries@agilent.com

Europe
info_agilent@agilent.com

Asia Pacific
inquiry_lsca@agilent.com

Find a local Agilent customer center in your country:
www.agilent.com/chem/contactus

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

RA45355.4045486111

Published in the USA, March 27, 2024
5994-7275EN

Agilent
Trusted Answers