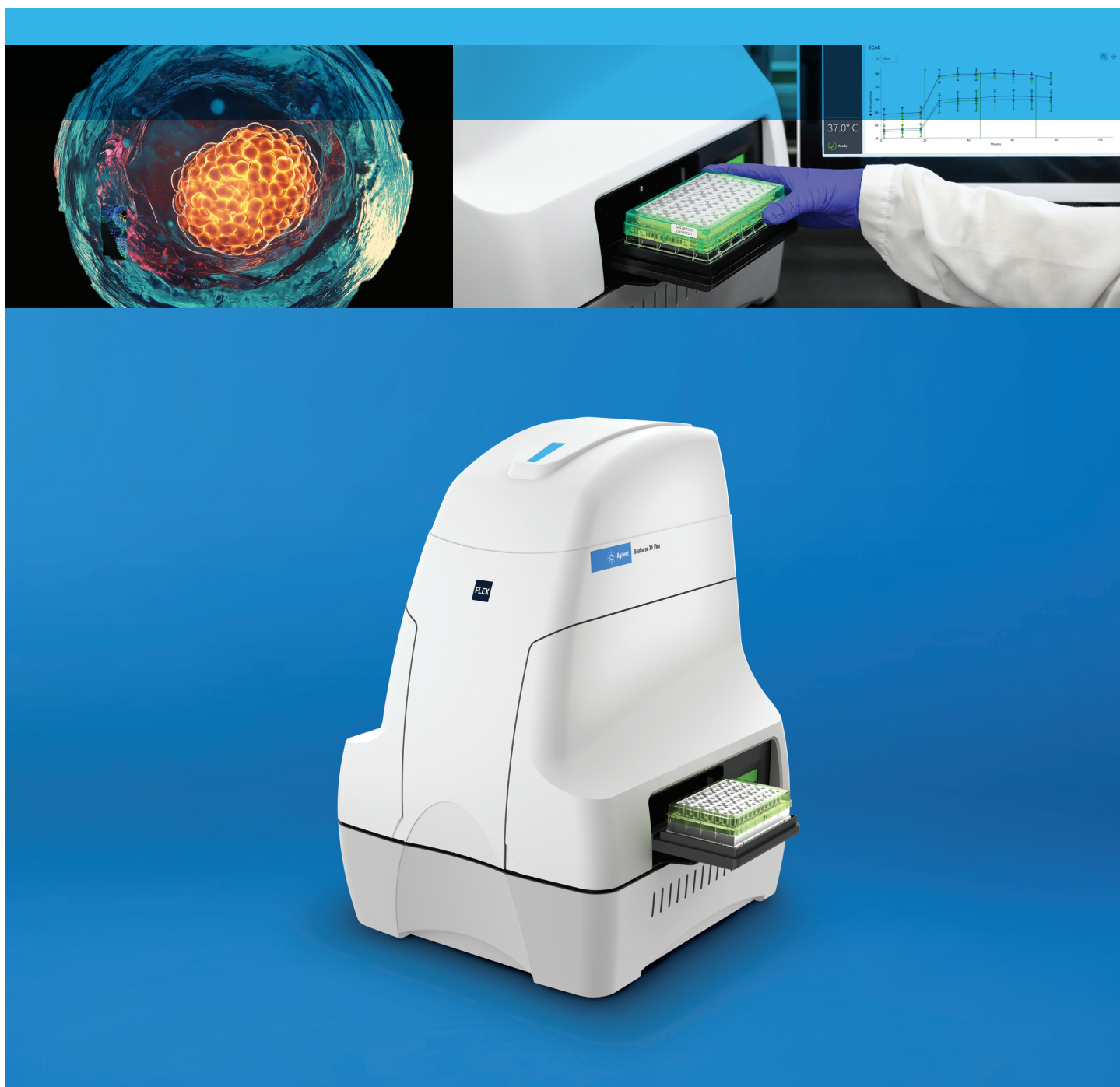
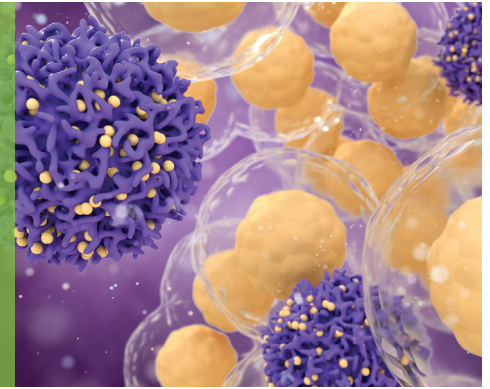


# Agilent Seahorse Extracellular Flux (XF) Flex Analyzer

Optimized for 3D model analysis and flexible for a wide range of biological models



# Discover the power of cellular energy metabolism



Metabolism is the backbone of cellular function, driving energy production alongside essential processes like biosynthesis, cellular adaptation, redox balance, mitochondrial function, and cell fate decisions. When disrupted, cells lose their ability to function properly, which contributes to disease. Analyzing metabolism provides deep insights into the processes that drive cellular activation, proliferation, differentiation, and death, allowing researchers to uncover disease mechanisms and open novel therapeutic avenues for unmet clinical needs.

Real-time, functional metabolic data is impacting research and therapeutic discovery in areas like these:

## **Cancer biology**

- Cancer cells rewire their metabolic programs to support rapid growth.
- Metabolic profiling provides insights on substrate requirements for cancer cell survival.
- Metabolic adaptations in tumor microenvironments help cancer cells evade treatment and death.

## **Obesity, diabetes, metabolic disorders**

- Metabolic dysfunction drives insulin resistance and diabetes progression.
- Imbalanced metabolism promotes chronic inflammation and accelerates obesity and metabolic disease.
- Altered/dysfunctional lipid metabolism plays a key role in metabolic disorders.

## **Neurodegeneration and aging**

- Mitochondrial dysfunction weakens neurons, leading to neurodegeneration.
- Metabolic imbalance drives oxidative stress, accelerating aging and cognitive decline.
- Glucose/lipid dysregulation severely impedes neuronal function.

## **Stem cell biology and regenerative medicine**

- Metabolic program and redox balance influences stem cell fate and tissue regeneration.
- Specific metabolic profile controls how stem cells remain in a self-renewing state.
- Proper metabolic function prevents premature aging.

## **Immunology**

- Metabolism drives immune cell lineage commitment.
- Metabolic pathways can be targeted to improve immunotherapy design and performance.

# Advance your research and therapeutic discoveries with deep insights and enhanced data translatability

The Agilent Seahorse XF real-time metabolic analyzer revolutionized the measurement of bioenergetics in live cells. This has provided the foundational knowledge about cell energy metabolism and its role in disease progression and therapeutic development. Building on our previous success, Agilent has developed a versatile system that expands real-time metabolic analysis beyond two-dimensional cell cultures, empowering physiologically relevant discoveries with three-dimensional models.

The 24-well Agilent Seahorse XF Flex analyzer features improved sensitivity and precision, a broad detection range, and dedicated consumables designed for optimized workflows compatible with 3D study models, such as tissue, organoids and coculture materials. Along with advanced experimental design and data analysis tools, you can now generate deeper scientific insights and enhance your data translatability, propelling your research into new frontiers of discovery.

## Versatile analyzer:

- Enhanced sensitivity and precision
- Wide range of detection
- Validated performance



## Smart plastics:

- Dual sensors ( $O_2$  and pH)
- Built-in injection ports
- Enhanced 3D capabilities



## Dedicated consumable for 3D studies

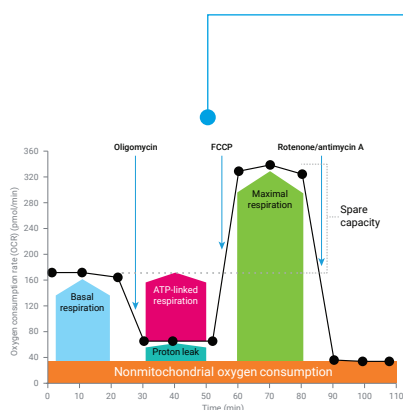
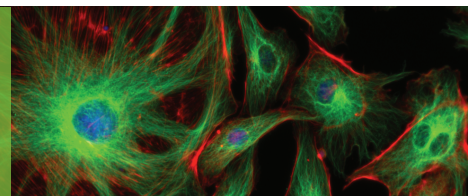
## State-of-the-art software for:

- Simplified assay setup
- Automated data QC
- Fast data transformation to insights



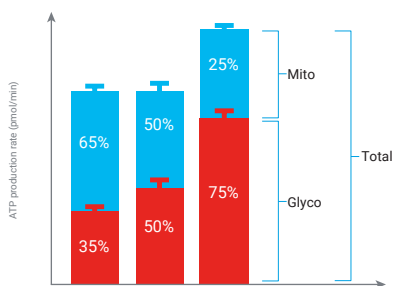
Optimized 3D workflows, and customized assay kit

# 2D cell culture applications



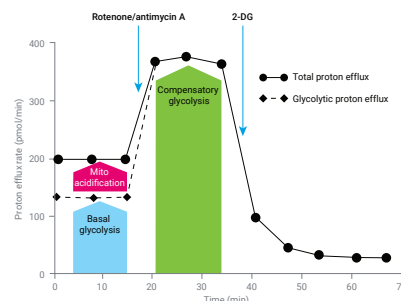
## XF Cell Mito Stress Test and Substrate Oxidation Stress Tests

- Get a complete mitochondrial respiration profile with multiparametric output.
- Determine fuel dependency for a specific cellular function or phenotype.
- Discover how manipulating substrate oxidation controls cell phenotype.



## XF Real-Time ATP rate assay

- Assess global cellular metabolic phenotype changes.
- Quickly determine bioenergetic pathway liabilities.
- Easily screen metabolic modulators.



## XF glycolytic rate assay

- Accurately quantify glycolytic activity in real time.
- Reveal kinetic insights not evident with an endpoint lactate accumulation assay.



For a full list of 2D applications, refer to [Agilent Seahorse XF Assay Kits and Reagents flyer](#).

## Enhance data quality

Integrate imaging and normalization into your Seahorse XF analysis for more consistent and interpretable results

The Agilent BioTek Cytation, an automated digital imaging system, can be seamlessly integrated into a Seahorse XF assay workflow when interfaced to the controller software for a single Seahorse XF. This system allows users to:

- Provide cell count per well and easily normalize signal by cell number to compare across treatment groups.
- Examine tissue and cell culture integrity/health before and after Seahorse XF assays using brightfield imaging.
- Improve data quality and interpretability.





# 3D applications



## Agilent Seahorse XF Flex tissue workflow

This optimized workflow incorporates the Agilent Seahorse XF Flex 3D microplate, Seahorse XF 3D Mito Stress Test kit, and a precision-cut vibratome, allowing for real-time metabolic analysis in live tissue models with greater sensitivity and consistency.

### Day before

#### Preparation

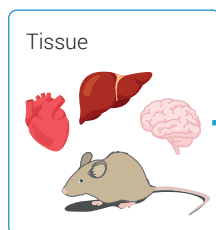
Hydrate a cartridge and prewarm Seahorse XF Flex 3D Capture microplate

Prepare slicing medium; chill overnight

Prepare assay template

### Day of assay

#### Dissect tissue of interest



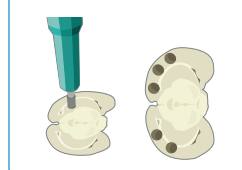
Prepare Seahorse XF assay media

#### Prepare tissue slice and punch



Prepare live tissue slices 150 – 200  $\mu$ m

Prepare tissue punches from tissue slices



#### Deliver tissue punch to well bottom and place ring/mesh

1. Place tissue punches into the well center of XF Flex 3D microplate; install mesh ring



2. Add medium; incubate microplate at 37  $^{\circ}$ C



#### Prepare compounds and perform Seahorse XF assay

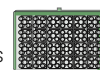
1. Prepare loading compounds

Contents	Amount/vial (nmol)
Oligomycin A	720
FCCP	540
Rotenone + Antimycin A	300



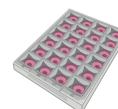
+ 3 mL/vial

2. Load cartridge ports

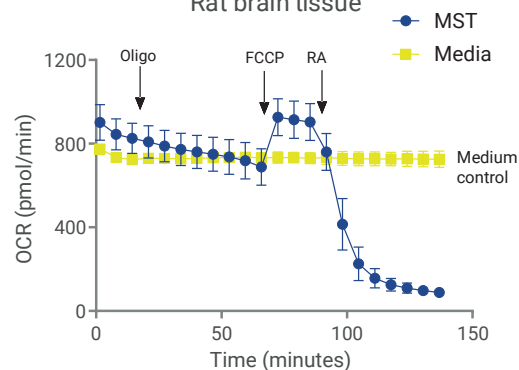


3. Start calibration

4. Load Seahorse XF Flex 3D microplate



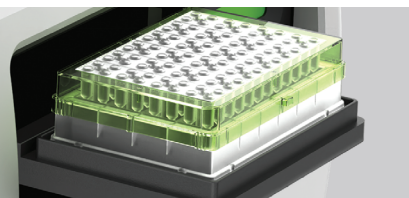
#### Rat brain tissue



Note: The Seahorse XF Flex Tissue workflow also supports investigation with small organisms and loose cell aggregate models, offering multifaceted, physiologically relevant approaches to studying cellular metabolism.

The Agilent Seahorse XF Flex Organoid Microplate and Workflow is coming soon.

# Specifications



<b>Dimensions</b>	38.74 cm W × 58.42 cm H × 45.72 cm D (15.25" W × 23" H × 16" D)
<b>Weight</b>	22.2 kg (49 lb)
<b>Power requirements</b>	100–240 VAC; 9 A; 50/60 Hz
<b>Operating environment</b>	Temperature: 4 to 30 °C Humidity: 20 to 80% Ambient O <sub>2</sub> : 3 to 21%
<b>Sample temperature</b>	16 to 42 °C (8 to 20 °C above ambient)
<b>Temperature uniformity</b>	+/- 0.5 °C
<b>Temperature accuracy</b>	+/- 0.7 °C
<b>Controller computer</b>	Dimensions: 53.34 cm W × 46 cm H × 30.48 cm D (21" W × 18" H × 12" D) Weight: 10 kg (22 lb) Operating system: Windows 11 Enterprise Memory: 16 GB Hard drive: 2 TB Screen display: Touch screen Communication: TCP/IP; USB; wireless
<b>Software</b>	Agilent Seahorse XF Flex controller, Seahorse XF Wave Pro (downloadable to a Windows computer) and Seahorse Analytics (web-based)

<b>Assay format</b>	24 well
<b>Signal range with verified performance</b>	OCR: 30 to 1050 pmol/min PER: 100 to 2500 pmol/min
<b>Low limit of detection (LLOD) with 95% confidence</b>	OCR: 30 pmol/min PER: 100 pmol/min
<b>Intraplate variation (standard deviation) at low rates*</b>	OCR: ≤ 30 pmol/min PER: ≤ 100 pmol/min
<b>Intraplate variation (coefficient of variation) at mid-high rates*</b>	OCR: ≤ 15% PER: ≤ 15%
<b>Recommended time to acquire one data point</b>	8 minutes
<b>Injection ports</b>	4 per well; 50 to 100 µL each
<b>Assay running volume</b>	500 to 1000 µL/well
<b>Sample requirements</b>	8,000 to 500,000 cells/well Also support tissue, organoids, and other 3D assay models

\* Low rates refer to signal range of 30 to 150 and 100 to 500 pmol/min for OCR and PER, respectively. Mid-high rates refer to signal range of 151 to 1050 and 501 to 2500 pmol/min for OCR and PER, respectively. Specifications are set for cultured 2D cells using the Agilent Seahorse XF V7 cell culture microplates.

## Powerful Agilent Seahorse software streamlines assay design and effortlessly transforms data into insights

The Agilent Seahorse XF Flex Controller and Wave Pro software includes preloaded templates, import features, and dose-response assay setup for quick design of experiments. Metabolic rate measurements are automatically calculated and displayed in real time, allowing you to observe live kinetic biological responses.

The **web-based Seahorse Analytics software** offers exclusive cutting-edge data analysis features (such as multile analysis, dose-response curves, and dedicated analysis views) that can turn data to insights quickly. It also provides secure data storage. Data can be exported into third-party graphing and statistical software (such as GraphPad Prism and Microsoft Excel).



# Take a close look at how XF technology works

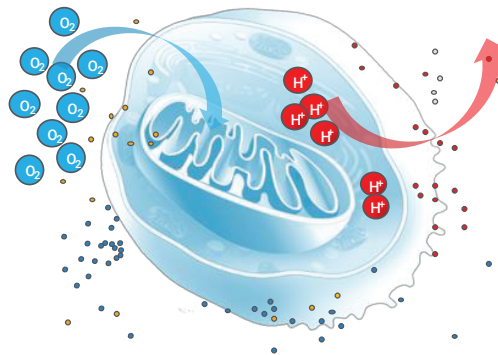
The Agilent Seahorse XF Flex analyzer simultaneously measures the activities of the two main energy pathways—mitochondrial respiration and glycolysis—in real time, using label-free, solid-state sensor cartridges in a 24-well plate format. It provides measurements that allow for a system-level view of cellular metabolic function in cultured cells and ex vivo 3D samples. The XF Flex analyzer works well with wide range of cell types, as well as tissue, organoids, and other 3D materials.

## Mitochondrial respiration: OCR (oxygen consumption rate)

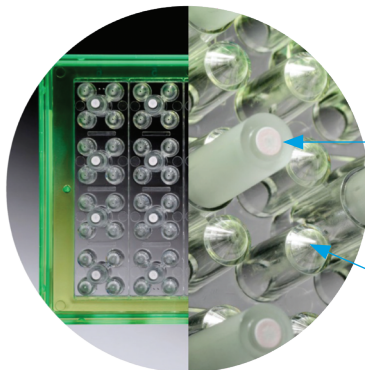
Mitochondria consume oxygen when oxidizing substrates to generate ATP. Mitochondrial respiration is measured by monitoring the OCR.

## Glycolysis: PER or ECA (proton efflux rate or extracellular acidification rate)

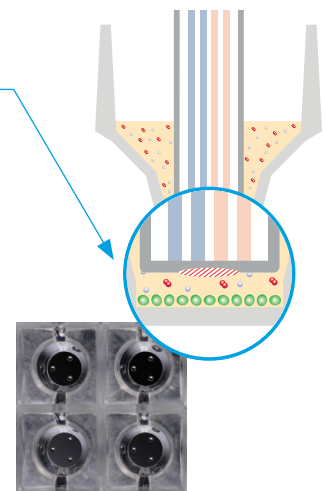
Cells generate ATP via glycolysis independent of oxygen, producing lactic acid and protons. The glycolysis is assessed by measuring PER or ECAR.



# Smart plastic technology makes it all possible



- Proprietary transient microchamber provides superior sensitivity and signal-to-noise ratio.
- Solid-state sensor probes contain polymer-embedded fluorophores, allowing detection of OCR and PER, or ECAR simultaneously.
- Patented integrated injection ports (four per well) for adding compounds, stimulators, inhibitors, substrates, and beads conjugated with antigens.



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