A Transformative Live-Cell Metabolic Assay Platform

Agilent Seahorse Extracellular Flux (XF) Pro analyzer
Drive Your Research and Therapeutic Discovery with a Platform Developed for Pharma and Better for Every Lab
The Agilent Seahorse XF analyzer revolutionized the measurement of bioenergetics in live cells in real time. Now, we've built upon that success with the Seahorse XF Pro platform. It combines enhanced instrument sensitivity and data consistency with intuitive custom workflow solutions and advanced experimental design and data analysis tools.

Agilent Seahorse XF data interpretation is even easier for characterizing *in vitro* disease models, identifying novel drug targets, validating target effect on cellular function, assessing drug safety, and measuring parameters linked to antitumor potential of T cell therapies. That means you can streamline your workflow from start to finish and generate and interpret metabolic data without the need for expertise in metabolism.

**Most advanced analyzer**
- High sensitivity
- Excellent OCR precision
- Validated performance
- Wide operation temperature

**State-of-the-art software**
- Easy assay setup
- Automated data QC
- Transforming data to actionable conclusions
- Enabling automation

**Smart plastics**
- Dual sensors (O₂ and pH)
- Built-in injection ports
- Reduced edge effect

**Validated kits, media, and reagents**

**Analytical Instrument Qualification service (AIQ)**
Discover the Power of Cellular Energy Metabolism

Energy metabolism plays a central role in a wide variety of cellular and physiological processes. How energy metabolism is programmed in cells not only can serve as a key indicator of cell health, but it also can be a powerful predictor of cell fate, function, and fitness. Such knowledge provides deep insights into the processes behind activation, proliferation, differentiation, and cell death, thus advancing science and assisting therapeutic discovery and development.
Live-cell, functional metabolic data is impacting therapeutic discovery and research in areas like these:

**Drug discovery**
- Discover/verify new therapeutic targets
- Ensure your cell models have the desired metabolic programs
- Identify mito toxicity to de-risk drug pipeline

**Immunology**
- Uncover the drivers behind cell fate
- Monitor/modulate immune cell activation in real time
- Target metabolic pathways to improve immunotherapy design and performance

**Cancer biology**
- Study how cancer cells rewire metabolic programs to support rapid growth
- Investigate which nutrients fuel cancer cells
- Reveal how the tumor microenvironment shapes metabolism

**Stem cell biology**
- Uncover the energy metabolic programs that drive stem cell pluripotency and differentiation
- Modulate medium conditions to achieve desired phenotype

**Cell therapy discovery and development**
- Evaluate engineering strategies, starting materials, and cell expansion conditions that can drive a metabolic phenotype linked to persistent T cells
- Understand how environmental conditions affect T cell metabolic fitness and exhaustion

**Obesity, diabetes, metabolic disorders**
- Measure functional outcomes of genetic changes to metabolic pathways
- Examine nutrient use, fatty acid oxidation, and glycolysis in different cell models: healthy versus diseased

**Other research areas**
- Explore the influences of energy metabolism that drive cell phenotype and function
Customized assays for cell therapeutics development

**XF T Cell Metabolic Profiling Assays** (Kit part number 103772-100)
With optimized reagents for different T cell populations, these assays provide robust bioenergetic parameters linked to two critical attributes for antitumor properties: T cell persistence and T cell 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 to maintain metabolic fitness in tumor microenvironments
- Can be used for NK cell metabolic profiling

Immune-cell activation assays for general research applications

Detect activation responses in minutes upon stimulation, providing an early window for studying immune cell activation, modulation, and related metabolic reprogramming.

**T Cell Activation Assay**
(Kit part number 103759-100)

**Macrophage Activation Assay**

**Neutrophil Activation Assay**
Core assays for therapeutic discovery and research

**XF Real-Time ATP Rate Assay** (Kit part number 103592-100)

This quantitative method measures the production rates of adenosine triphosphate (ATP) from mitochondrial respiration and glycolysis simultaneously in live cells. It can be used to:

- Assess metabolic phenotype changes during cell activation, proliferation, and differentiation.
- Identify pathway liabilities.
- Screen for compound effect on cellular ATP production.

**XF Cell Mito Stress Test** (Kit part number 103015-100)

Use this widely recognized test to gain a comprehensive understanding of mitochondrial (dys)function.

- Report on multiple parameters—including basal and ATP-linked respiration, maximal and spare respiratory capacity, and proton leak.
- Investigate functional differences between cell types and the impact of genetic or pharmaceutical interventions.

**XF Glycolytic Rate Assay** (Kit part number 103344-100)

Accurately measure glycolysis in live cells by quantifying proton efflux rate (PER) specific to glycolysis.

- Detect transient and rapid metabolic switches (such as the Warburg effect) in minutes.
- Measure the effects of metabolic modulators on glycolytic rates.
- Report on multiple key parameters: basal and compensatory glycolysis.

**XF Substrate Oxidation Stress Tests**

(Kit part numbers 103572-100, 103573-100, 103574-100)

With these optimized kits, you can dive deeper to study the impact of three primary substrates on mitochondrial and cellular function.

- Explore which substrates are relevant or required for a specific cellular function and/or phenotype.
- Discover how cell phenotype can be controlled via manipulation of substrate oxidation activities.
Dysfunctional metabolism is associated with many different disease states and is a rich source for bolstering target discovery programs. By incorporating live-cell metabolic measurements, early drug discovery scientists are gaining deeper understanding of therapeutic target effects and the impact of therapeutic agents to alleviate disease states.

**Here’s what scientists are saying:**

“The newly designed hardware minimizes the edge effects [and] improves analysis accuracy and reproducibility (95% data with CV <10%). [It also] doubles the number of compounds I can test on each 96-well plate.”

— Yili Xu, Senior Associate Scientist, Gilead Sciences

**Discover how XF Pro technology can advance your drug discovery studies**

Dysfunctional metabolism is associated with many different disease states and is a rich source for bolstering target discovery programs. By incorporating live-cell metabolic measurements, early drug discovery scientists are gaining deeper understanding of therapeutic target effects and the impact of therapeutic agents to alleviate disease states.

- Provide cellular attributes linked to antitumor potential of T cell therapeutic products
- Identify novel drug targets
- Validate target effect on cell function
- Confirm function post genetic modification
- Characterize in vitro disease model
- Determine drug safety
Take a close look at how XF technology works

The Agilent Seahorse XF Pro analyzer simultaneously measures the activities of the two main energy pathways—mitochondrial respiration and glycolysis—in live cells using label-free, solid-state sensor cartridges in a 96-well plate format. It provides measurements that allow for a system-level view of cellular metabolic function in cultured cells and ex vivo samples. The XF Pro analyzer works with most cell types, including primary cells, adherent cell lines, and suspension cells.

**Glycolysis: PER**
Cells generate ATP via glycolysis independent of oxygen, producing lactic acid and protons. The XF Pro analyzer measures glycolysis by measuring the proton efflux rate (PER) of cells.

**Mitochondrial respiration: OCR**
Mitochondria consume oxygen when oxidizing fatty acids or other substrates to generate ATP. The XF Pro analyzer measures mitochondrial respiration by measuring the oxygen consumption rate (OCR) of cells.

**Smart plastic technology makes it all possible**

Patented integrated injection ports (4 per well) for adding compounds, stimulators, inhibitors, substrates, and beads conjugated with antigens let you test multiple conditions per well.

Solid state sensor probes contain polymer embedded fluorophores that are inspected through a machine vision process, allowing detection of OCR and PER simultaneously.

Patented transient microchamber provides superior sensitivity and signal to noise ratio.
Enhance data quality and transform data into insights

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

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

- Easily normalize signal by cell count to compare across treatment groups for adherent cell types
- Examine cell culture integrity/health before and after XF assays using bright field imaging
- Improve data quality and interpretability

Intuitive, streamlined experimental setup and data analysis experience empowered by the state-of-the-art XF software and analytics platform

Wave Pro Controller software, created for the XF Pro analyzer, includes preloaded templates, advanced template import features, and dose-response assay setup for quick design of experiments.

Web-based Seahorse Analytics offers exclusive cutting-edge data analysis features (such as multi-file analysis, hit identification from screening assays, dose-response curves, Z-prime) allowing for customizable analysis views/reports that can turn data to insights quickly. It also provides secure data storage. Data can be exported into third-party graphing and statistical software like GraphPad Prism and Microsoft Excel.
### Specifications for the Agilent Seahorse XF Pro

<table>
<thead>
<tr>
<th>Specifications</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimensions</td>
<td>15.25'' W × 23'' H × 16'' D (38.74 cm W × 58.42 cm H × 45.72 cm D)</td>
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<tr>
<td>Weight</td>
<td>49 lbs (22.2 kg)</td>
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<tr>
<td>Power requirements</td>
<td>100 to 240 VAC; 9 A; 50/60 Hz</td>
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<tr>
<td>Operating environment</td>
<td>Temperature: 4 to 30 °C</td>
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<tr>
<td></td>
<td>Humidity: 20 to 80%</td>
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<tr>
<td>Sample/assay temperature</td>
<td>16 to 42 °C</td>
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<tr>
<td>Temperature uniformity</td>
<td>0.5 °C</td>
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<tr>
<td>Temperature accuracy</td>
<td>0.7 °C</td>
</tr>
<tr>
<td>Operational environment O(_2) range*</td>
<td>3 to 21%</td>
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</tbody>
</table>

**Controller computer**
- Operation system: Microsoft Windows 10
- Memory: 16 GB
- Screen display: Touch screen
- XF software: Full instrument control, assay design, and data viewing capability
- Communication: TCP/IP; USB; wireless
- Dimensions: 21'' W × 18'' H × 12'' D (53.34 cm W × 46 cm H × 30.48 cm D)
- Weight: 22 lbs/10 kg

**Analysis software**
- XF Wave Pro: Can be downloaded to a Windows laptop or desktop computer
- Seahorse Analytics: A web-based analysis platform

**Assay format**
- Live cells in a specially designed 96-well microplate

**Verified performance range**
- OCR: 13 to 350 pmol/min
- PER: 50 to 950 pmol/min

**Low limit of detection (LLOD) with 95% confidence**
- OCR: 13 pmol/min
- PER: 30 pmol/min

**Intraplate variation (standard deviation) at low rates***
- OCR: ≤7 pmol/min
- PER: ≤16 pmol/min

**Intraplate variation (coefficient of variation) at mid-high rates***
- OCR: ≤10%
- PER: ≤15%

**Recommended time to acquire one data point**
- 6 minutes

**Maximal recommended assay**
- Up to 6 hours

**Injection ports**
- 4 per well; 20 to 30 µL each

**Assay running volume**
- 160 to 300 µL/well

**Sample requirements**
- Typically 5,000 to 250,000 cells/well

**Plate material**
- Tissue-culture treated polystyrene or poly-D-lysine coated polystyrene

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* This supports studies under hypoxia conditions.

** For signals above the range, the system may also provide linear measurements. However, performance was not verified.

*** Low rates refer to signal ranges of 13 to 70 and 30 to 210 pmol/min for OCR and PER, respectively. Mid-high rates refer to signal ranges of 71 to 350 and 210 to 950 pmol/min for OCR and PER, respectively.

### Product information

<table>
<thead>
<tr>
<th>Product</th>
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</thead>
<tbody>
<tr>
<td>Seahorse XF Pro Analyzer with XF Discovery license</td>
<td>S7855A</td>
</tr>
<tr>
<td>Seahorse XF Pro Analyzer with XF Discovery license and XF Imaging and Normalization solution</td>
<td>S7855AN</td>
</tr>
</tbody>
</table>
Agilent Value Promise

We guarantee you at least 10 years of instrument use from your date of purchase. Otherwise, we will credit you with the residual value of the system toward an upgraded model.

Learn more:
www.agilent.com/chem/discoverxf

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