Application Note

Cell Analysis

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Trusted Answers

A Superior System for Real-Time Metabolic Analysis with Brain Tissue and Other 3D Models

Authors

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Abstract

Neuronal development and function are among the most energy demanding biological processes in the body. The brain requires a constant, ready source of energy to function properly, relying on both mitochondrial respiration and glycolysis to continually supply ATP to meet this demand. Disruption of oxygen metabolism and mitochondrial function are consistent pathological features of various aging and neurodegenerative diseases associated with cognitive decline. Different brain regions and structures have differing functions and energy demands. To study mitochondrial metabolism in the brain, researchers often use cultured neurons or isolated mitochondria from brain tissue. These techniques require large amounts of tissue and do not capture the complex tissue microenvironment, or the interplay of the multiple cell types present in brain tissue.

In this application note, we introduce the Agilent Seahorse XF Flex analyzer together with a streamlined workflow that allows for detection and quantitative assessment of energy metabolism in small biopsy punches of distinct brain regions. We provide examples of metabolic data from brain tissue samples using the Agilent Seahorse XF Flex 3D Capture Microplate-L and the Agilent Seahorse XF 3D Mito Stress Test kit. In addition, techniques for preparing and optimizing brain tissue used in the Seahorse XF assays and analyzing data are discussed.

The Agilent Seahorse XF platform is an integrated label-free solution that seamlessly combines the Seahorse XF analyzer, sensor cartridges, assay kits, and software to deliver real-time functional metabolic data. This platform provides key parameters, such as oxygen consumption rate (OCR) and extracellular acidification rate (ECAR), which are vital indicators of mitochondrial health, toxicity, glycolysis, and overall cellular (dys)function.

Introduction

The demand for tools and reagents for the analysis of 3D cellular models is growing rapidly. Precision tissue slices, organ-on-a-chip, bioprinting, and organoids are key examples of these 3D models. Precision tissue slices are noteworthy because they are accessible, inexpensive, and include supportive cell types, preserving the natural environment of the tissue. Tissue samples are used extensively in research and drug screening due to their ability to mimic the in vivo environment and their ability to provide a more accurate representation of physiology compared to traditional 2D cell cultures.[1,2,3,4]

The Agilent Seahorse XF analyzer is the gold standard method for assessing metabolic function. It measures oxygen consumption rate (OCR) and extracellular acidification rate (ECAR), which reflect mitochondrial respiration and glycolysis, respectively. While this technology has been fundamental for understanding how metabolism affects cellular functions and drives disease progression, the data obtained mostly used two-dimensional cultured cells or isolated mitochondria, and may not fully capture the complex environment of tissue.

To address this, researchers have developed ex vivo methods to measure mitochondrial respiration using tissue slices including brain. These approaches provide a physiological representation of the cellular environment, including intercellular coordination and metabolic coupling among different cell types. Understanding brain bioenergetics has evolved from a simplistic view to a dynamic model of metabolic reprogramming and adaptation. This involves mitochondria utilizing various energy substrates and allocating ATP production between glycolysis and oxidative phosphorylation. Studies have shown metabolic shifts in glial cells upon inflammatory activation in aging and pathological conditions.[5,6,7,8] These studies provide insights into brain tissue workflows, but they employ a variety of Seahorse XF instrumentation and consumables that have not been optimized for tissue.

In this application note, we introduce a new Seahorse XF system for the interrogation of energy metabolism in 3D models using brain tissue slices as an example. This system incorporates the Seahorse XF Flex 3D Capture Microplate-L, the Seahorse XF 3D Mito Stress Test kit, a vibratome tissue slicer, and the Seahorse XF Flex analyzer (Figure 1).

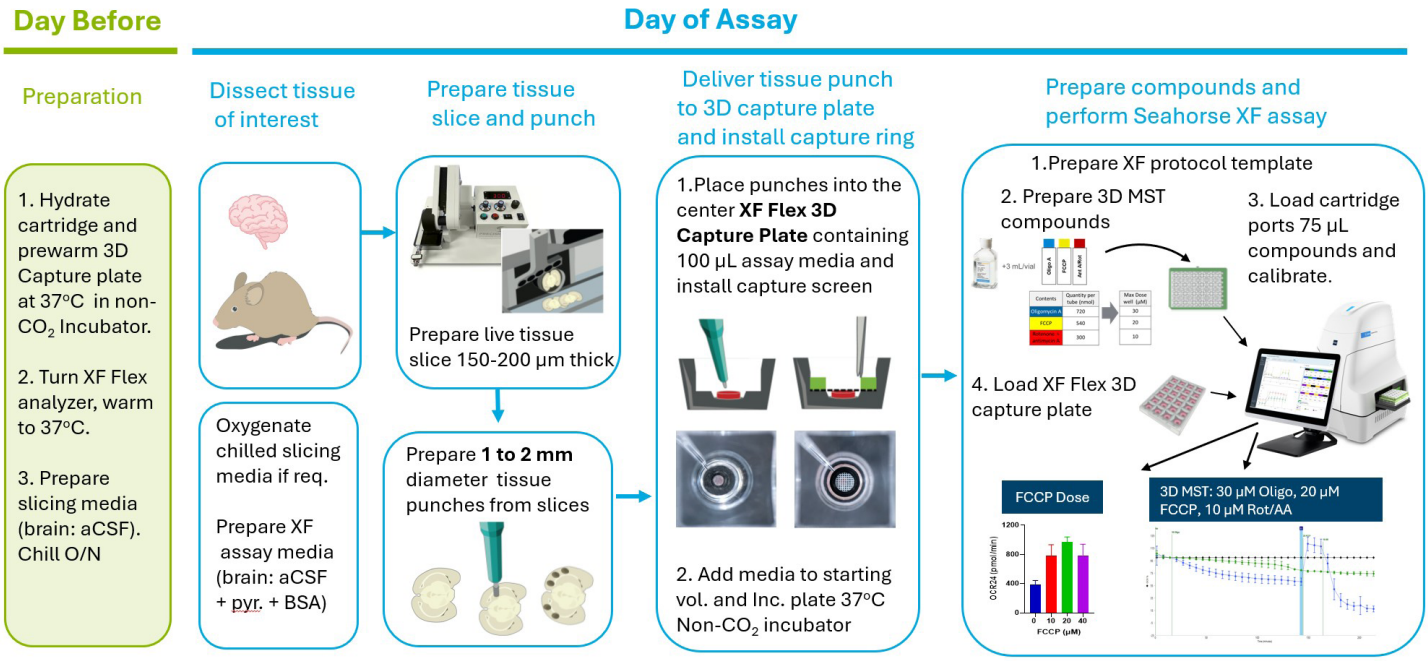


Figure 1. The Agilent Seahorse XF Flex 3D tissue workflow illustration, highlighting the key steps in the assay.

The Seahorse XF Flex analyzer features improved sensitivity and precision with advanced experimental design and data analysis elements. The 3D capture plate-L is a 24-well plate and has a sample chamber at the bottom of each well that is 250 µm deep and 3.15 mm in diameter. These dimensions allow for the analysis of tissue slices of the same or smaller sizes. The plate is also provided with a capture ring for each well, which is designed to hold tissue material in place and can form a transient microchamber of 8.5 µL when measurements are taken. The mesh on the capture ring has a large pore size, allowing for sufficient reperfusion of gases and chemicals, as well as recovery of medium conditions during Seahorse XF assays. The Seahorse XF 3D Mito Stress Test kit contains modulators of the electron transport chain and supports study models that require high doses, such as tissue samples and small organisms. The system provides enhanced sensitivity, reproducibility and improved usability.

Experimental

Tissue preparation and set up of the Seahorse XF Flex 3D Capture Microplate-L

Nine-week old female Sprague Dawley rats were obtained from Taconic Biosciences (SD-F, MPF health status) and acclimated several days at Hooke Laboratories where the rat brain was dissected on the morning of the Seahorse XF assays. After dissection, rat brains were immediately placed in chilled and oxygenated artificial cerebral spinal fluid (aCSF) buffer (Table 1) and delivered to the Agilent laboratory, in Lexington, MA, where the tissue was prepared for Seahorse XF assays.

Table 1. Artificial cerebral spinal fluid composition. Adjust the buffer pH to 7.4 and store at 4 °C. Oxygenate for 1 hour before use.

Component	Concentration (mM)
NaCl	120
KCl	3.5
$CaCl_2$	1.3
Mg_2Cl_2	1.0
KH_2PO_4	0.4
HEPES	5
D-glucose	10

Before processing the tissue, 100 mL aCSF supplemented with 0.6 mM pyruvate, 100 mL Seahorse XF aCSF supplemented with 0.6 mM pyruvate and 0.4% fatty acid free BSA, and 20 mL aCSF supplemented with 7.5 mM pyruvate, pH 7.4 (see Table 2A, 2B, and 2C, respectively), were warmed up. The pH was adjusted to 7.4 at 37 °C and filter sterilized before use. 100 µL of the buffer in Table 2A was added to each well in the 3D capture plate-L and set aside until tissue punches were ready.

Table 2A. Agilent Seahorse XF aCSF assay media with 0.6 mM pyruvate (no BSA).

Component	Final (mM)	Stock Solution (mL)
aCSF Buffer		198.8
100 mM Pyruvate	0.6	1.2
pH	7.4	

Table 2B. Agilent Seahorse XF aCSF assay media with 0.6 mM pyruvate and 0.4% BSA.

Component	Final (mM)	Stock Solution (mL)
aCSF Buffer		99.4
100 mM Pyruvate	0.6 mM	0.6
200 mg/mL BSA	4 mg/mL	2.0
pH	7.4	

Table 2C. Agilent Seahorse XF aCSF assay media with 7.5 mM pyruvate (no BSA).

Component	Final (mM)	Stock Solution (mL)
aCSF Buffer		18.5
100 mM Pyruvate	7.5	1.5
pH	7.4	

Upon arrival, the brain tissue was sliced using a Compresstome VF-510-0Z Vibrating Microtome (Precisionary Instruments, LLC) in chilled and oxygenated aCSF buffer, following the manufacturer's recommendations (also see Figure 2 for key steps). The Compresstome settings used to prepare the brain slices were Speed 2 and Oscillation 4. Tissue slices were transferred to a 6-well plate or petri dish containing oxygenated aCSF buffer and with a punch pad (TedPella; catalog number 15087-1) placed at the bottom of each well or petri dish. Tissue punches were then produced using a biopsy punch tool of desired size (TedPella; catalog number 15110-10, 15110-15, or 15110-20), and delivered to the 3D capture plate-L containing 100 µL buffer per well.

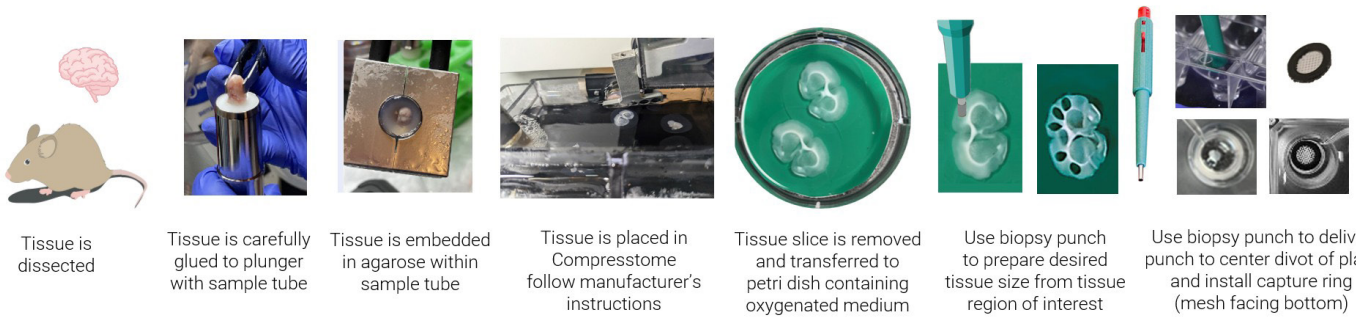


Figure 2. Illustration of key steps involved in the preparation of rat brain tissue slices, using the Compresstome from Precisionary Instruments, LLC.

No tissue was placed in wells A1 and D6 as they were used as background wells. Tissue punches were centered in each well. Then, one 3D capture ring was placed in each well, including background wells, with mesh side down and pushed firmly down to the well bottom. Each well was then topped up with the buffer in Table 2B (with BSA) to a total volume of 600 µL. It was ensured that no bubbles were trapped in the wells. Then the 3D capture plate-L with tissue samples was placed in a 37 ºC non-CO_2 incubator for 45–60 minutes before the assay.

Perform the Seahorse XF 3D Mito Stress Test

Seahorse XF assays were performed following the instructions described in the Agilent Seahorse XF 3D Mito Stress Test kit User Guide, including preparation of compounds, reagents, and sensor cartridges. The Seahorse XF buffer in Table 2A was used to prepare oligomycin A and rotenone/antimycin A injection solutions and the Seahorse XF buffer in Table 2C was used to prepare FCCP injection solution. The Seahorse XF 3D Mito Stress Test template provided in the Agilent Seahorse Wave Controller software was used and adjusted when needed. This template is optimized for tissue samples with default setting of a 3-minute mix, 0-minute wait, and 3-minute measure for each cycle. The number of cycles is set to 3 for basal, 15 for oligomycin A, 3 for FCCP, and 8 for rotenone/antimycin A (Figure 3).

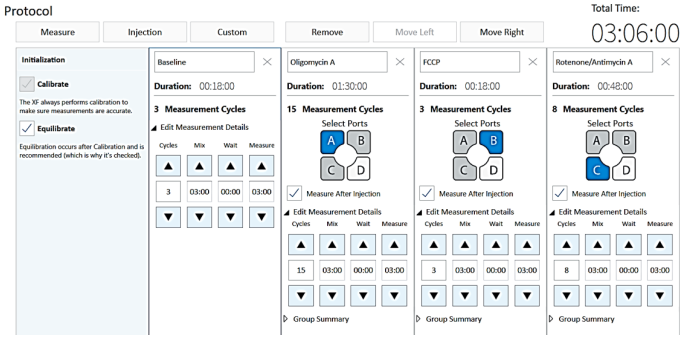


Figure 3. Agilent Seahorse XF 3D Mito Stress Test template in the Agilent Seahorse XF Flex Controller software.

Protein quantification

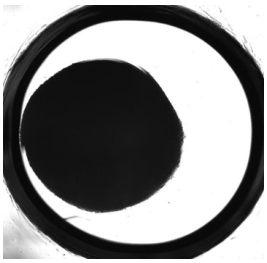
At the conclusion of the Seahorse XF assay, medium was removed from each well of the 3D capture plate-L, followed by three washes with PBS to remove the BSA present in the aCSF buffer. The background wells were also washed and used as background controls for the protein assay. After the third wash, 100 µL of RIPA lysis buffer (Thermo Scientific) containing protease inhibitor (Thermo Scientific) was added directly to the bottom of each well. The plate was then wrapped in parafilm and frozen at –80 ºC overnight. The plate was thawed and tissue slices were homogenized by pipetting the RIPA buffer through the mesh several times until tissue was lysed. Next, the plate was centrifuged at 250 g for 10 minutes and 25 µL of lysate was transferred in duplicate to a 96-well assay plate. This was done carefully with an electronic pipet so that residual volume remained in pipet and no air or bubbles were introduced to 96-well assay plate. A BSA standard curve was prepared and 25 µL of each standard was added to assay plate along with background controls.

Total protein content was determined using the Pierce BCA Assay (Thermo Scientific) following manufacturer's instructions. Briefly, 200 µL of prepared assay reagent was added to each well and plate was covered with a plate seal, and mixed on a plate shaker for 0.5–1 minute. The plate was incubated at 37 ºC for 30 minutes and absorbance at 562 nm was read using an Agilent BioTek Synergy H1 multimode reader. The standard curve was used to determine the total protein content of each unknown sample, which was used to normalize Seahorse XF assay data.

Determination of tissue surface area

The Agilent BioTek Cytation 1 cell imaging multimode reader was used to acquire high-contrast brightfield images of the tissues in the 3D capture plate-L prior to the installation of the capture ring. Gen5 software was used to determine the average diameter or area for each tissue disc (see illustration in Figure 4).

A.



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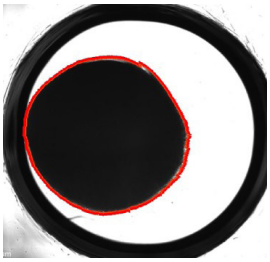

Figure 4. Representative well images with the methods of area determination. The inner black area indicates the location of tissue sample. (A.) Example using the measurement tool within the Agilent BioTek Gen5 software to determine the diameter of the tissue sample in both X and Y axis. (B.) Example of Gen5 area measurement in red which automatically determines diameter and area of sample but only if the tissue samples are in center of well and not impacted by the edge of the sample chamber (visible black ring).

XF 3D Mito Stress Test data analysis

Analysis and data transformation of the Seahorse XF 3D Mito Stress Test result files were performed using Seahorse Analytics, a web-based software platform. Using kinetic OCR measurements, dedicated features (widgets) in Seahorse Analytics automatically calculated key 3D Mito Stress Test assay parameters.

Materials and equipment

Materials	Vendor	Part Number
Seahorse XF Flex 3D Capture Microplate-L	Agilent Technologies	103864-100
Seahorse XF 3D Capture Screen Insert tool		101135-100
Seahorse XF 3D Mito Stress Test kit		103016-100
Seahorse XF 1.0 M glucose solution		103577-100
Seahorse XF 100 mM pyruvate solution		103578-100
Seahorse XF Flex analyzer		S7851A or S7851AN
Cytation 1 imaging system		
Artificial Cerebral Spinal Fluid (aCSF)	Made fresh within 48 h of assay day, pH 7.4, sterile filtered and stored at 4 ºC. Oxygenate for 1 hour before use.	
Compresstome VF-510-0Z Vibrating Microtome	Precisionary Instruments	VF-510-0Z
Crazy glue		VF-VM-GLUE-CANAL
2% Agarose tablets		VF-AGT-VM
1, 1.5, and 2 µm Tissue punches with plungers	TedPella	15110-10, 15110-15, 15110-20
Cutting mat		15087-1
RIPA lysis buffer	Thermo Scientific	89900
Protease inhibitor		87786
Pierce BCA Assay		23225

Results and discussion

Assay optimization requisition

To effectively examine metabolic and bioenergetic function effectively using Agilent Seahorse XF analyzers, it is essential to first determine the appropriate amount of tissue material required to perform Seahorse XF assays and the optimal concentrations for all modulators.

The 3D capture plate-L has a sample chamber of 250 µm deep by 3.15 mm in diameter at the bottom of each well. Therefore, tissue sizes should not exceed 250 µm in thickness and 3 mm in diameter. It is recommended to leave some space in the sample chamber to allow sufficient gas and nutrient exchange between the tissue and medium.

During assay optimization, 1, 1.5, and 2 mm diameter brain tissue discs with 200 µm thickness were tested using the Seahorse XF 3D Mito Stress Test kit. As shown in Figure 5, the punch sizes showed linear relationship to OCR values, indicating the sizes in this range can be appropriate or acceptable to move forward.

To determine the optimal size, the O_2 and proton level data must be examined using a full Mito Stress Test (MST), essentially to include the full range of the signals in an assay. In general, the starting basal O_2 level should be around 152 mmHg, and not below 100 mmHg. Also, at the start of each measurement, the O_2 level must be fully recovered, and at the end of each measurement, the O_2 level should not fall below 40 mmHg. This is because extended period of exposure to extremely low O_2 levels (hypoxia) can have adverse impact on tissue health. In addition, the pH level should be within the linear range for the sensors between pH 7.5 (+/- 0.1) to 6.5 (+/- 0.1). Outside this range, measurement accuracy can be compromised.

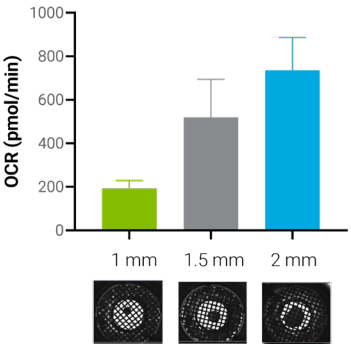

Figure 5. OCR signal correlates to tissue punch sizes. The images below the chart were taken using an Agilent BioTek Cytation 1 cell imaging multimode reader, showing the tissue discs under the capture ring in the well.

For the Seahorse XF Flex analyzer, the instrument protocol for mixing height and speed is optimized for the 3D capture plate-L, allowing medium condition to fully recover to the original state at the beginning of each measurement. Figure 6 is an example of acceptable data, showing O_2 levels of around 130 mmHg at the start of the assay and full O_2 level recovery at the beginning of each measurement (Figure 6A). The pH level data also falls withing the range of 7.5 (+/- 0.1) to 6.5 (+/- 0.1) (Figure 6B).

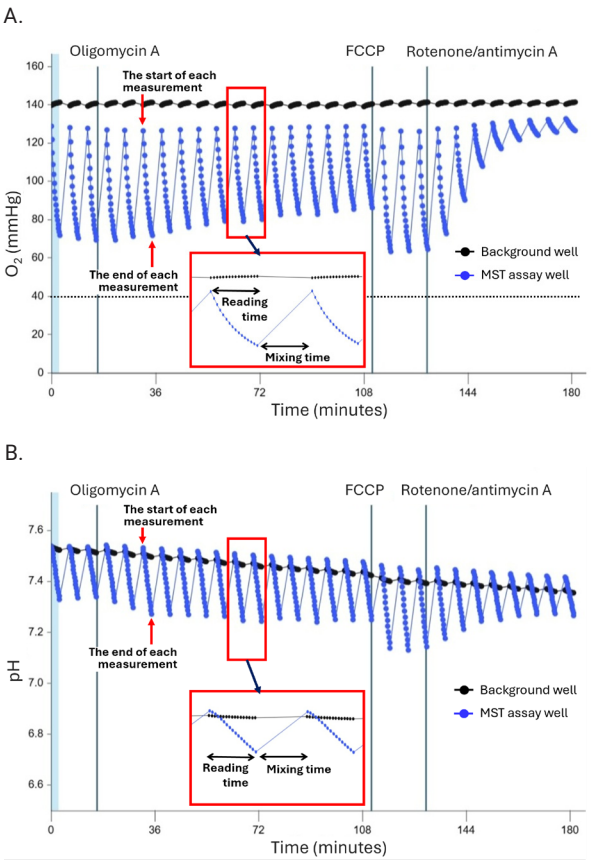

Figure 6. Examples of appropriate levels of oxygen consumption (A) and pH level data (B).

The optimal modulator concentration for obtaining robust results is tissue dependent and a titration experiment for each modulator must be performed for each tissue type during assay optimization. This titration is especially important for FCCP as too low or too high concentrations can lead to diminished response and underestimation of the maximal respiratory capacity. Buffer conditions can also affect optimal modulator concentration, such as inclusion of BSA or serum in assay medium. Therefore, an optimization experiment is also needed when medium conditions are changed. For high respiring tissue types, higher concentrations of substrate, such as pyruvate and glucose, may be added in the FCCP injection buffer to minimize substrate depletion during assay.

Figure 7 is an example of an FCCP concentration optimization experiment using the Seahorse XF 3D Mito Stress Test kit and rat cortex tissue discs, 200 µM thick and 2 mm in diameter. In this test, all samples were injected with oligomycin A at a final concentration of 30 µM following three baseline measurements. FCCP at varying concentrations was injected after twenty measurements post oligomycin A injection. The assay buffer contains approximately 0.32% BSA. The data shows that all FCCP concentrations increased respiration, and the highest OCR is observed at 20 µM. Therefore, the optimal concentration for FCCP is determined to be 20 µM.

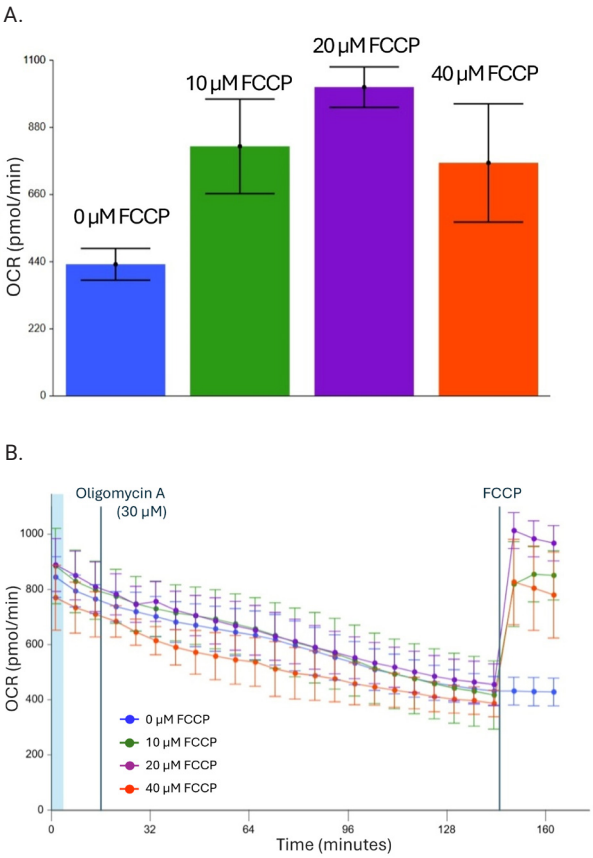

Figure 7. FCCP optimization. All experimental groups were injected with oligomycin A at 30 µM final concentration after three basal measurements as indicated in the kinetic graph. A. OCR at measurement 24, the first measurement after FCCP injection. Concentration indicated in the graphs are final concentrations in the well. B. OCR Kinetic graph. n=5-6.

Optimization experiments for oligomycin A and rotenone/antimycin A were also performed (data not shown). Based on the results, it was determined that the optimal concentrations for oligomycin A and rotenone/antimycin A were 30 µM and 10 µM, respectively.

System performance evaluation

The results of a Mito Stress Test for rat brain tissue, using the new system incorporating the 3D capture plate-L, the 3D MST kit, and Seahorse XF Flex analyzer and following the optimized conditions described in the previous section is presented in Figure 8. As shown, a robust basal OCR and decent responses kinetics to all modulators were observed (Figure 8A). All key parameters indicating mitochondrial function were also obtained (Figure 8B).

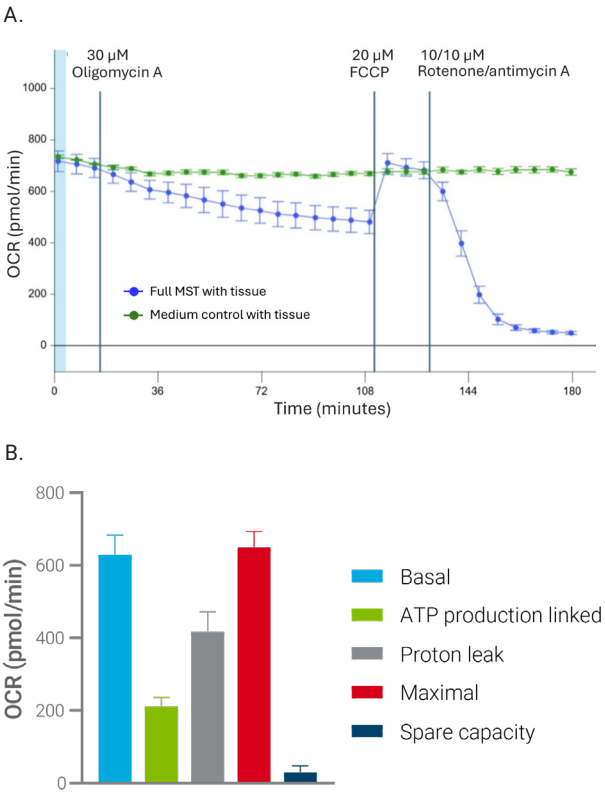

Figure 8. Agilent Seahorse XF Mito Stress Test with rat brain tissue discs (200 µm × 2 mm) performed using the 3D capture plate on XF Flex analyzer. A. OCR Kinetic graph. B. Key parameters of mitochondrial function. n=12 MST wells. n=3 tissue control wells.

In contrast, the same assay performed with the Seahorse XF islet capture microplate on the Seahorse XFe24 analyzer showed very small or no responses to modulators (Figure 9). This side-by-side comparison clearly demonstrates the improved sensitivity and data robustness provided by the new system that incorporates the 3D capture plate-L and Seahorse XF Flex analyzer. The data also shows that the response to oligomycin A is much slower than the typical response observed in cultured 2D cells, therefore, it is recommended to incorporate a minimum of 10 measurements post oligomycin A injection. It is also recommended that a tissue control (without adding modulators) is added for each assay to monitor the stability of respiration signals throughout the assay during assay optimization.

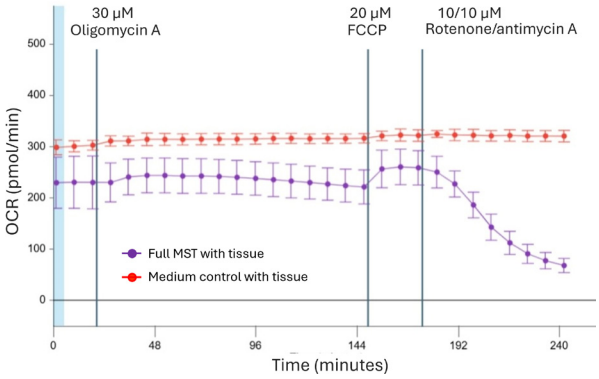

Figure 9. OCR kinetic graph of the Agilent Seahorse XF Mito Stress Test with rat brain tissue discs (200 µm × 2 mm), performed using the Agilent Seahorse XF islet plate on the Agilent Seahorse XFe24 analyzer. n=12 MST wells. n=3 tissue control wells.

Tissue preparation considerations

To perform a successful Seahorse XF real-time metabolic assay, is it crucial to use tissue materials that remain healthy and viable for hours to ensure functionality throughout the assay. Although, conditions suitable for sample preparation may vary depending on tissue types, cold temperatures are often used. For instance, brain and liver tissues are usually handled in ice-cold aCSF or Krebs-Henseleit Buffer (KHB), respectively, which is oxygenated with either 100% O_2 or 95% O_2:5% CO_2. These conditions can potentially reduce the impact of injury from slicing. Adipose tissue, on the other hand, can be prepared at room temperature. Regardless, the tissue materials should be prepared as soon as possible after dissection. It is advisable to consult with experts who are experienced in handling the specific tissue type for other assay types.

It is also important to obtain uniform tissue sizes to achieve consistent assay results. Although the literature has reported several methods to generate small tissue pieces, such as using surgical scissors, biopsy punch tools, and various types of tissue cutters, we recommend the use of a vibratome tissue slicer, such as the Compresstome VF-510-0Z from Precisionary Instruments LLC, together with a biopsy punch tool. This method can quickly produce precise tissue samples in large quantities while minimizing cellular damage. The Compresstome VF-510-0Z is easy to use and can generate viable tissue slices with consistent thickness in the 150 to 200 µm range, which is the thickness range recommended for performing Seahorse XF assays. It is found that for some tissue type, such as brain tissue, allowing tissue slices to rest or acclimate at room temperature in oxygenated buffer before punching the tissue into discs can help with data consistency.

Following tissue slicing, a biopsy punch tool is employed to generate consistent tissue samples or discs. Tissue discs from desired tissue regions can be selected for studying spatial differences in metabolism. Biopsy punch tools with built-in plungers are preferred as they allow easy delivery the samples to the center of the sample chambers in the 3D capture plate-L. A soft pad under the tissue slices is also recommend when punching the slices, as this allows for a clean cut while forcing the tissue disc into the plunger, so the tissue disc can be easily transferred and delivered to the sample chambers in the 3D capture plate-L. In addition, general-use paintbrushes can assist in the handling of delicate tissue slices and discs. Soft bristles help prevent damage to tissue slices during transfer.

The working volume for the 3D capture plate-L ranges from 600 to 1000 µL per well. However, we recommend starting with an initial volume of 50 to 100 µL of medium in each well before placing tissue samples. This small volume helps to keep the tissue sample in the well center and facilitates the installation of the 3D capture ring. After the installation of the capture ring, it is recommended to capture images of the tissue samples in the wells using a brightfield imaging system (4×), to check for bubbles. Bubbles trapped under the mesh may lead to abnormal O_2 and pH signals, resulting in highly variable rate data. Bubbles should be eliminated before performing a Seahorse XF assay. The images also help to evaluate tissue placement, ensuring all wells have tissue samples as intended. Figure 10 is a brightfield image of a 3D capture plate-L loaded with tissue and capture rings, showing that in most wells, tissue disc placement was appropriate, except for well A3.

Half of the tissue disc in this well is pinched under the ring. Examination of the OCR signal shows that the OCR value from this well is less than 50% of the average value for the plate.

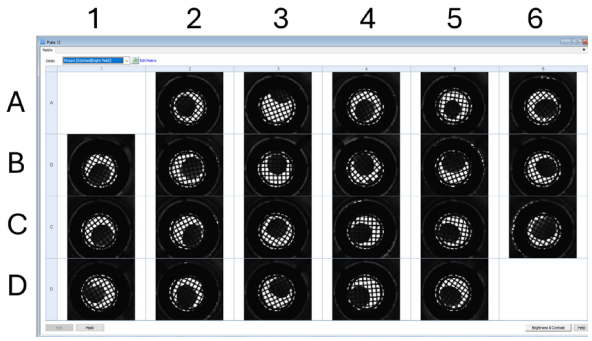

Figure 10. Brightfield Images of Agilent Seahorse XF Flex 3D Capture Microplate-L. 4× BFHC montage images of 2 mm discs of rat brain cortex tissue from 200 µm slice, after 3D capture ring installation.

Data analysis and normalization options

Analysis of Seahorse XF metabolic assay data can be performed using the Agilent cloud-based software application, the Seahorse Analytics. It provides desktop-like interactivity with an intuitive interface and is available at any time and from anywhere in the world. A 3D Mito Stress Test kit companion view is available with widgets specifically designed for assays with 3D models. This companion view provides the option of data analysis without oligomycin injection.

Normalization of Seahorse XF metabolic assay data is important in interpretation of assay results. There are several options that can be considered for 3D samples, including total protein, sample size or weight, and percentage of baseline signal (baselining). The optimal method, however, depends on the 3D models being studied, study objectives, as well as assay conditions (such as medium composition).

Quantification of total protein can be a convenient method as tissue samples in the assay plate can be stored in lysis buffer at –80 ºC for days after the Seahorse XF metabolic assay. It is important that tissue material is properly lysed. If assay medium contains BSA or serum, a washing step is required. Figure 11 compares the data normalized with total protein and the not normalized data. As shown, two samples in the blue experimental group exhibited reduced OCR values throughout the assay.

After normalizing to total protein, the OCR values were adjusted to similar levels as other samples in the same group. Keep in mind that this method not only measure intracellular protein content but also is affected by the amount of extracellular matrix protein present in tissue materials.

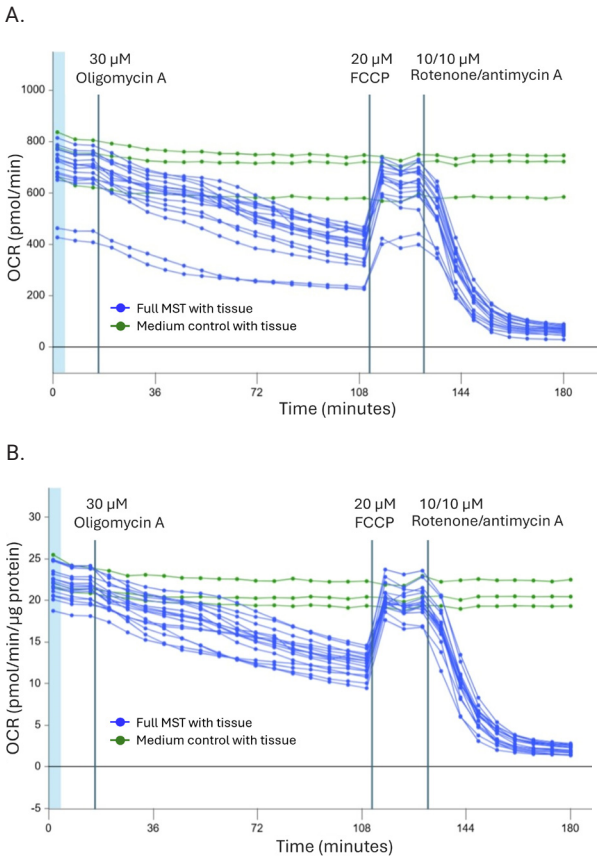

Figure 11. OCR kinetic graph of an Agilent Seahorse XF Mito Stress Test with rat brain tissue discs (200 µm × 2 mm), performed using the 3D capture plate-L on the Agilent Seahorse XF Flex analyzer. Each line represents an individual well. A. Not normalized data. B. Normalized data based on total protein per well.

Normalization by sample size/area or weight can also be readily achieved by automated imaging systems. It is commonly used when methods of generating consistent tissue samples are not available. Figure 12 is an example of data normalized to tissue surface area. This is the exact same data from Figure 5 where it shows that the OCR values range from 190 to 730 pmol/min among sizes of 1, 1.5, and 2 mm. The normalized data shows that OCR values per unit of area among these tissue sizes are within 30%.

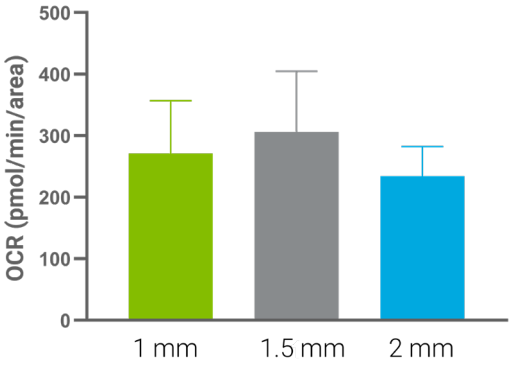

Figure 12. OCR signals normalized to sample area, using the un-normalized raw data from Figure 5.

In some cases, Seahorse XF assay data can be normalized by baseline signal or any measurement in the kinetic traces. So, all other measurements are expressed as a percentage of the baseline signal. This method is applicable when a comparison of responses to an acute treatment to modulators is needed. It is especially useful when tissue materials vary among replicates within the same experimental group.

Figure 13 is an example of the baselined result of OCR kinetic graph using the same raw data from Figure 8A. As shown, the error bars for all the data points are smaller compared to Figure 8A, and the data is much cleaner than the original not baseline data. This method can be easily performed using the "Baseline" button in the Seahorse Analytics or in the Wave software.

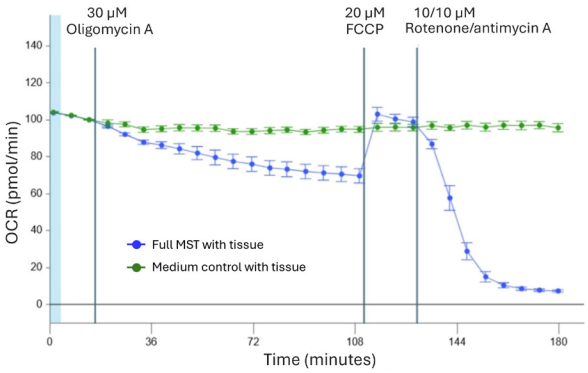

Figure 13. Baselined OCR kinetic graph of Figure 8A.

Conclusion

Agilent Seahorse XF technology revolutionized bioenergetic measurements in live cells by enabling metabolic analysis in real time. This enabling technology has been fundamental for gaining the understanding of how metabolism drives disease progression and helping advance therapeutic development.

The Seahorse XF Flex analyzer along with purpose-designed 3D capture plate-L expands this revolutionary system beyond traditional two-dimensional cell cultures, empowering physiologically relevant discoveries with three-dimensional models, such as tissues materials. It is demonstrated with brain tissues that this system provides the necessary sensitivity to gain robust tissue respiration signal and clear responses to mitochondrial modulators, making it a superior tool for real-time metabolic analysis in tissues. With the guidance discussed in this application note, metabolic interrogation of brain tissue can be achieved with greater success and minimized effort. This system and streamlined workflow can also be easily adapted for other tissue types and organisms in a wide range of biomedical research applications.

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Products used in this application

Agilent products

Agilent Seahorse XF Flex Analyzer

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