

Islet Assay Using the Agilent Seahorse XF24 Islet Capture Microplate

Technical Overview

Introduction

Agilent Seahorse XF Analyzers are most commonly used with an adherent monolayer of cells attached to an Agilent Seahorse XF Cell Culture Microplate. Many researchers desire using more physiologic cellular sources such as primary pancreatic islets, which are routinely used to study diabetes. Agilent Seahorse Bioscience, in collaboration with the Mitochondrial Advancing Research through Collaborations (ARC) at Boston University School of Medicine, has developed a protocol that uses a novel consumable, the Agilent Seahorse XF24 Islet Capture Microplate, to assess whole islet bioenergetics *in vitro*. The advantages over traditional methods (for example, Clarke Electrode Apparatus) include higher throughput (20 sample per assay) and smaller amounts of pancreatic islets (as low as 30 islets per well).



Materials and Methods

The assay workflow (Figure 1) describes the procedure used to prepare reagent, materials, and injected compounds. The whole islet protocol described below is a modification of the Seahorse XF24 Analyzer Protocol described in the Agilent Seahorse XF24 User Manual (Version 1). Modify the protocol to realize your intended research goals.

Modified Agilent Seahorse XF Assay Media (MA Media): Supplement XF DMEM assay media with 3 mM glucose and 1 % FBS to run whole islets. FBS is needed to prevent the islets from becoming too adherent.

Note: When planning a Pancreatic Islet assay, Agilent recommends using an anti-adherent for accurate reproducible results. Please contact Agilent Technical Support with any questions.

It is recommended that all compounds to be added or injected are diluted into MA Media, as described in Table 3.

Other items needed

- Agilent Seahorse Islet Capture FluxPak (p/n 101174-100)
- Agilent Seahorse XF24 Islet Capture Microplate (p/n 101122-100)

- Islet Capture Screens
- Agilent Seahorse XF Islet Capture Screen Insert tool (p/n 101122-100)
- Agilent Seahorse XF Calibrant Solution (p/n 100840-000)
- Dissecting microscope
- Open faced bio-hood
- Multichannel pipettes and tips
- Eppendorf and 15/50 mL Falcon tubes

Table 2. Components/formulation of modified Agilent Seahorse XF Assay Media.

Compound	Brand	Catalog number	MW or molar concentration	Final concentration	Grams or mL for 500 mL of XF Assay Media
Glucose	Sigma	G7528	180	3 mM	0.27 g
FBS	Hyclone	SH30070.03	100 %	1 %	5 mL

Table 3. Dilutions of modified Agilent Seahorse XF Assay Media.

Compound	Brand	Catalog number	Final concentration	Dissolve in:
Rotenone	Sigma	R8875	5 µM	Stock 1,000x in DMSO Dilute to 10x in MA Media
Oligomycin	Sigma	O4876	5 µM	Stock 1,000x in DMSO Dilute to 10x in MA Media
FCCP	Sigma	C2920	1 µM	Stock 1,000x in DMSO Dilute to 10x in MA Media
Glucose	Sigma	G7528	20 mM	Stock 1,000x in DMSO Dilute to 10x in MA Media
FBS	MP Biomedicals	155765	5 µM	Methanol

Note: Oligomycin, FCCP, rotenone, and myxothiazol should be freshly diluted in MA Media for each experiment. Stock solutions in DMSO may be stored at -20 °C.

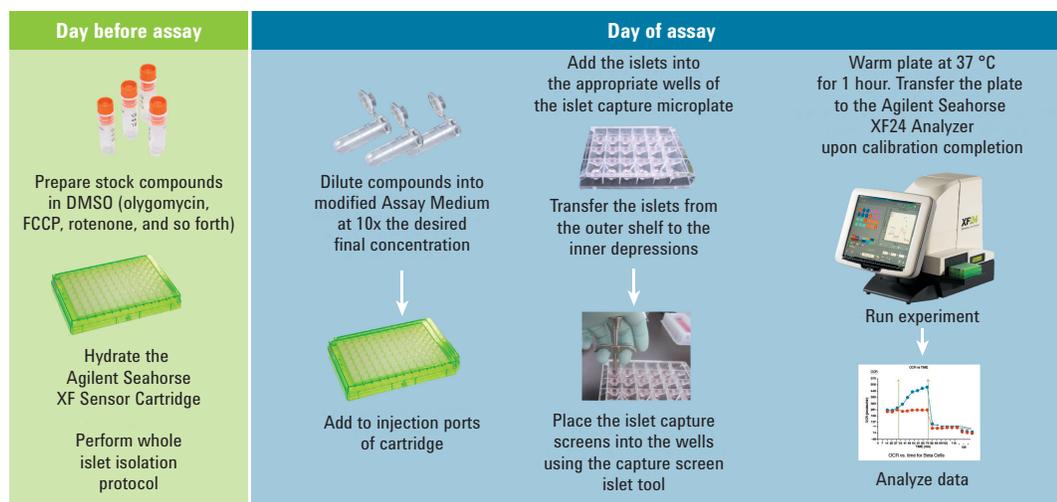


Figure 1. Pancreatic islet assay workflow

Day Before Assay

1. Prepare an XF Assay Template (using the Assay Wizard).
 - a. Use the Agilent Seahorse XF24 Operation Manual as a guide and incorporating proper experimental design.
 - b. Upload the assay template to the Seahorse XF24 Analyzer before starting the assay. This experiment is an example of how to obtain the various mitochondrial respiration states using the Seahorse XF24 Analyzer.
 - c. Use Table 4 as a guide to program the Mix-Wait-Measure and Injection protocol.
2. Prepare the Agilent Seahorse XF Sensor Cartridge.
 - a. Hydrate the Seahorse XF Sensor Cartridge overnight in Seahorse XF Calibration Buffer at 37 °C, without CO₂.
 - b. Prepare whole islets by the standard protocol(s) used in your laboratory. For this protocol, ~eight mice were sacrificed to obtain ~1,400 islets: enough for 20 wells at 70 islets/well. Incubate whole islets in a petri dish overnight under standard conditions for islet culture. (For the data shown, islets are cultured in RPMI media with 11 mM glucose, 10 % FBS, and 1 % pen/strep.)

Day of Assay

1. Add whole islets and capture screens to the wells.
 - a. Aspirate islets from a petri dish, and dispense them into a 50-mL tube.
 - b. Wash 1x in MA Media.
 - c. Remove supernatant, and resuspend in 2 mL MA Media.
 - d. While creating turbulence in the tube with a 20 µL pipettor, take 20 µL aliquots and place as a drop on a culture dish; make three drops total (provides ~3 % of the islets).
 - e. Count islets under a dissecting microscope. This provides an average amount of islets per volume, from which you can estimate the total number of islets.
 - f. Determine the count of the islets, and adjust the volume to obtain ~70 islets for every 100 µL of media (700 islets/mL).
 - g. Add 400 µL of MA Media to each well of the XF24 Islet plate.
 - h. Add 50 µL of the islet suspension to each well, and repeat so that each well gets a total of 100 µL of the islet suspension. The final volume should be 500 µL per well.
 - i. When islets are seeded, use a 20 µL pipette to move all of the islets onto the depressed chamber in the bottom of the well.

This step is tedious – use a dissecting microscope to be sure all of the islets are in the depression at the bottom of the well, as in Figure 2.

Table 4. Typical mix and measurement cycle times for XF24-3 assays.

Command	Time (minutes)	Port
Calibration	–	–
Equilibrate	12*	
Mix	2	
Wait	2	
Measure	3	
Mix	2	
Wait	2	
Measure	3	
Mix	2	
Wait	2	
Measure	3	
Inject A		
Mix	2	
Wait	2	
Measure	3	
Mix	2	
Wait	2	
Measure	3	
Mix	2	
Wait	2	
Measure	3	
Inject B		
Mix	2	
Wait	2	
Measure	3	
Mix	2	
Wait	2	
Measure	3	
Mix	2	
Wait	2	
Measure	3	

* The default Equilibrate command consists of 2 minutes Mix, 2 minutes Wait, repeated 3x. The same pattern could be followed for more injections.

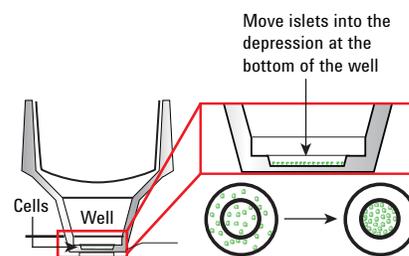


Figure 2. Islets should be in the depression at the bottom of the well.

2. Add screens by prewetting them in MA Media in a small petri dish to remove any air bubbles (Figure 3).

a. Use a pair of sterile forceps to position the screens so that the ring is facing up. (Figure 3)

b. Use the capture screen insert tool to pick up an islet capture screen from the petri dish.

c. Carefully, place the islet capture screen in the bottom of each well using the capture screen insert tool (Figure 4).

d. Take care during this step that you do not cause too much turbulence. Keep the islets resting in the depression at the bottom of the well.

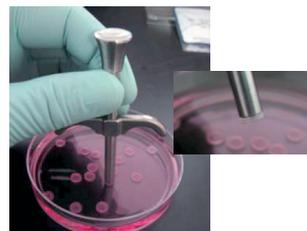
e. Release the islet capture screen into the well by pulling up on the T-lever on the capture screen insert tool.

f. Ensure the islet capture rings are stuck firmly at the bottom of the well. This can be confirmed by gently pushing the screen down with a blunt pipette tip. (Figure 5)

g. Ensure that there is an islet capture screen in each well, even if there are no cells in the well. A microplate without a full complement of screens will cause problems with the head on the Seahorse XF24 Analyzer.



Face-up: Prewetting of screens to remove air bubbles.



Use islet capture screen insert tool.

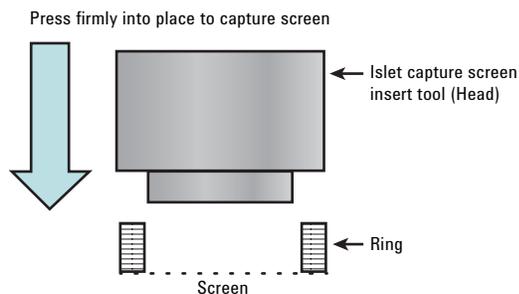
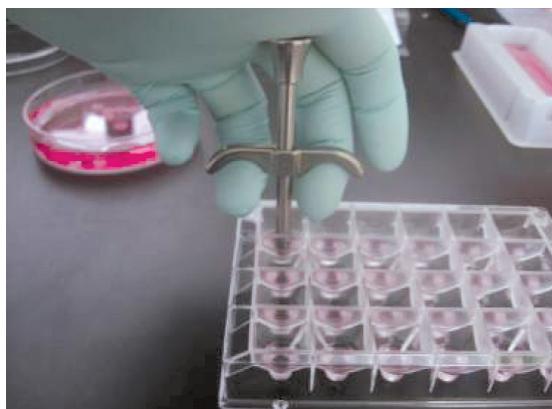


Figure 3. Steps outlined in Day of Assay.



Carefully place the screen in the well, creating as little turbulence as possible.

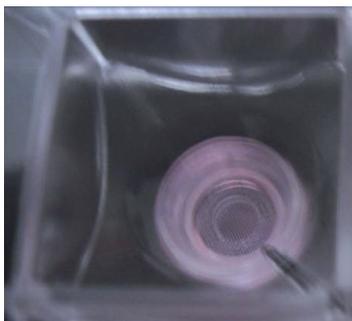


Pull up on the T-lever to release the capture screen once it is in place.

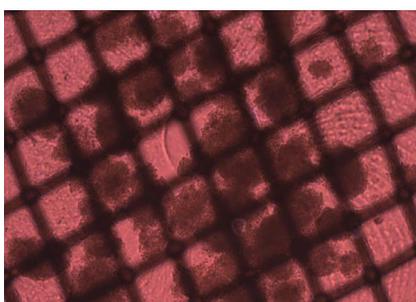
Figure 4. Day of Assay: Place islet capture screen.

3. Run the Seahorse XF24 Islet Capture Microplate on the Seahorse XF24 Analyzer.

- a. Place the microplate in an incubator set at 37 °C, without CO₂.
- b. Store the microplate in the incubator for at least 1 hour to equilibrate the temperature, and adjust islet metabolism to 3 mM glucose.
- c. While the plate is incubating, load the Agilent Seahorse XF Sensor Cartridge injection ports with the compounds listed in Table 5.
- d. After the cartridge is filled with compounds for injection, load the cartridge and start the program and calibration (as described in the Agilent Seahorse XF24 User Manual).
- e. When the Seahorse XF24 Analyzer calibration is complete, place the islet plate into the Seahorse XF24 Analyzer. Run the program.
- f. Following completion of the program, data can be normalized using the number of islets per well or basal rates. Islets may also be harvested for further downstream analysis, for example, protein quantification.



Well with screen in place



Islets under screen

Figure 5. Day of Assay: Checking the screen placement.

Table 5. Agilent Seahorse XF Sensor Cartridge injection port compounds table.

Injection ports	Volume	Concentration in port	Final concentration in well
Glucose	50 µL	200 mM	20 mM
Oligomycin	55 µL	50 µM	5 µM
FCCP	60 µL	10 µM	1 µM
Rotenone	65 µL	50 µM	5 µM
Antimycin A	65 µL	50 µM	5 µM

Note: Vigorous mixing of the stock 20 µM oligomycin is required to prevent precipitation. Rotenone and antimycin A are mixed together in the appropriate concentrations for injections.

Data Analysis

The results in Figures 6 and 7 were obtained using 70 mouse islets/well or isolated beta cells.

Figures 8 and 9 show a direct comparison between normal human islets and diabetic human islets. Note that the basal OCR readings for the normal islets are 4x higher than that of diabetic islets, and that the response to glucose is depressed in the diabetic islets compared to the normal islets.

Notes, Suggestions, and Comments

The methods described above have been used successfully with whole pancreatic islets isolated from both mouse and humans. We believe that whole islets from other species can be used by following this protocol, however, the tissue, species (including age and sex), and method of isolation will contribute to the overall activity and other variables associated with the whole islets.

Starting values, ranges, and optimization

It is recommended that the following parameters be explored and optimized depending on the overall goal(s) of the experiment and research topic.

- Amount of whole islets per well
- The concentration of substrates and compounds injected
- Mix, Wait, and Measure times

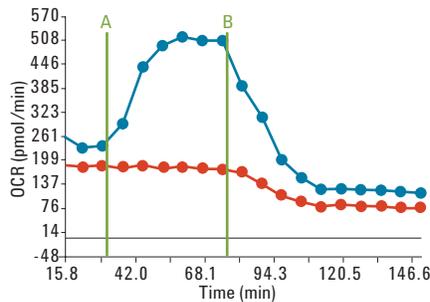


Figure 6. Oxygen consumption rate versus time for whole islets.

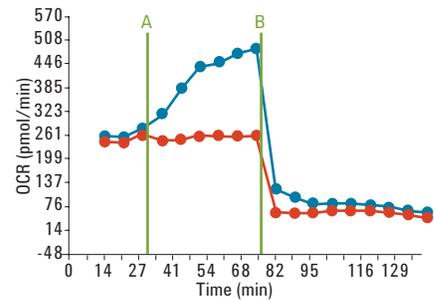


Figure 7. Oxygen consumption rate versus time for beta cells.

Whole pancreatic islets show a similar response to glucose addition as clonal INS1 beta cells run in a standard XF culture plate. Red lines: Blank injection at A, oligomycin at B. Blue lines: 20 mM glucose injection at A, oligomycin at B. *Unpublished data from the Shirihai lab at Boston University School of Medicine.

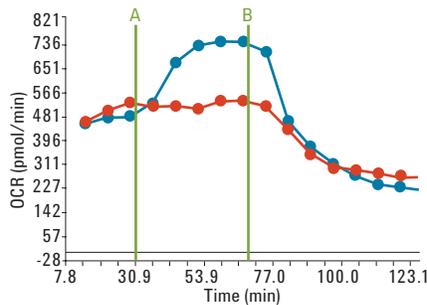


Figure 8. Oxygen consumption rate versus time for normal human islets.

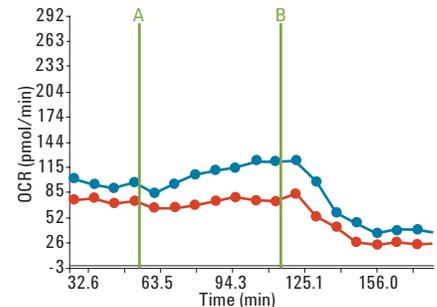


Figure 9. Oxygen consumption rate versus time for diabetic human islets.

Comparisons of normal human islets versus diabetic human islets run in the Islet Capture Microplate. Red lines: Blank injection at A, oligomycin at B. Blue lines: 20 mM glucose injection at A, oligomycin at B. *Unpublished data from the Shirihai lab at Boston University School of Medicine.

Reference

Please see the Agilent Seahorse XF24 Training Course Workbook for a complete guide to operating and analyzing data used in the Agilent Seahorse XF24 Analyzer.

For methods on isolating whole islets, please see: <http://www.jove.com/video/255/murine-pancreatic-islet-isolation>

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