



Performing XF Assay

Analyzing real-time bioenergetics

Seahorse XF^e analyzer

海馬生物能量代謝測定儀

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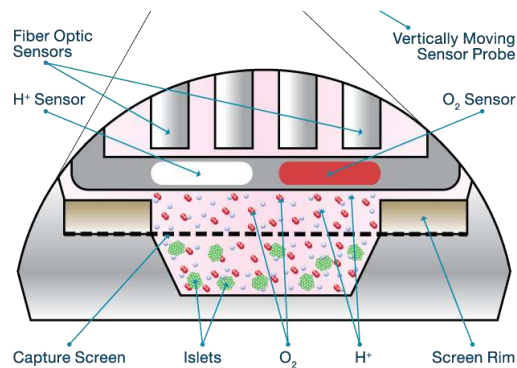
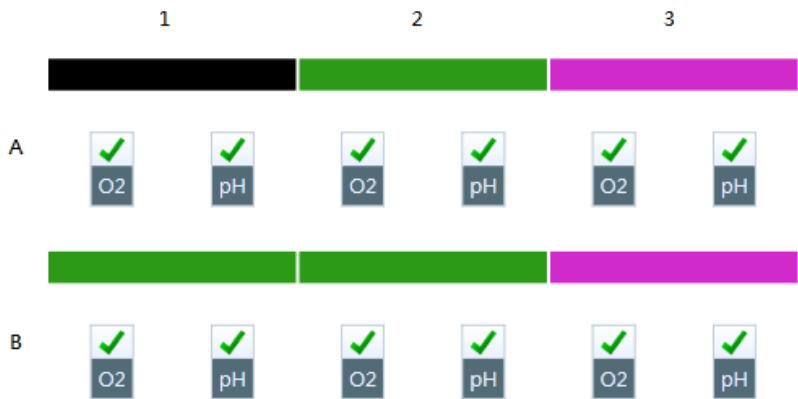
Consumables



XF²⁴ FluxPaks



Islet FluxPaks



High Stability
High Reproducibility

Kits



Mito Stress Test Kit

- ✓ *Oligomycin*
- ✓ *FCCP*
- ✓ *Rotenone & Antimycin A*



Glycolysis Stress Test Kit

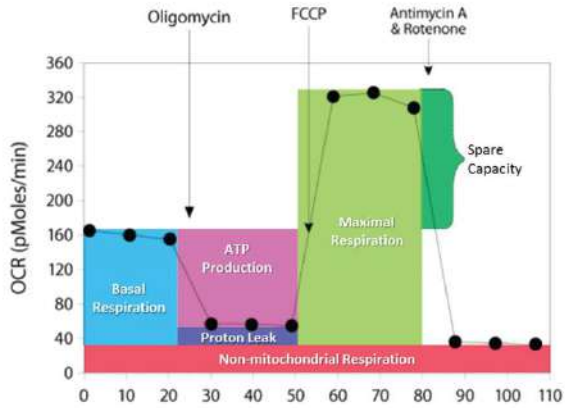
- ✓ *Glucose*
- ✓ *Oligomycin*
- ✓ *2-DG*



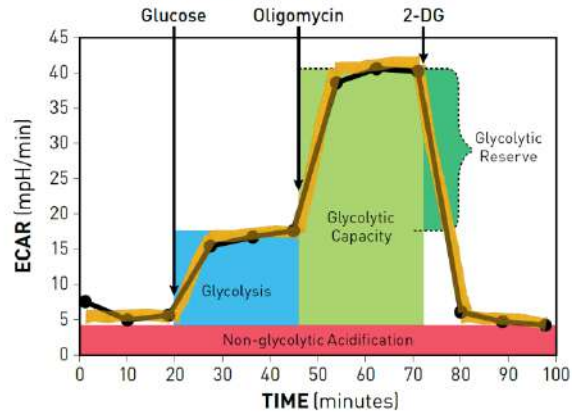
Palmitate-BSA FAO Substrate

- ✓ *BSA*
- ✓ *Palmitate-BSA*

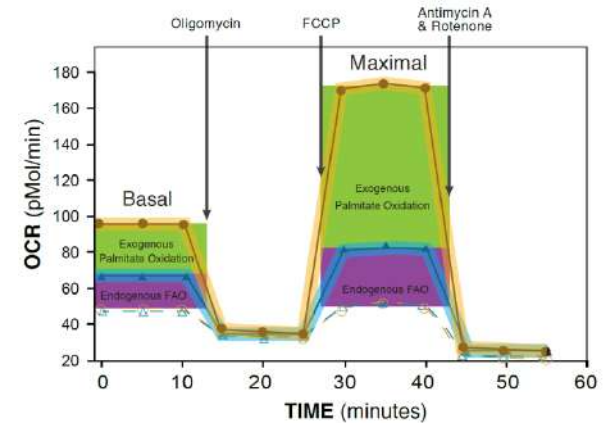
Mitochondrial Respiration



Glycolytic Function



Exogenous Palmitate Oxidation & Endogenous Fatty Acid Oxidation



Media

- **Culture media V.S. Assay media**

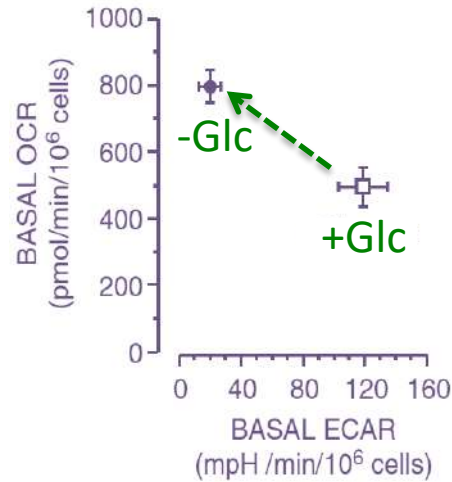
- Sodium bicarbonate
- HEPEs
- Serum (2%)

- **Nutrient**

- Glucose (Low, High)
- Sodium Pyruvate
- Glutamine

- **pH**

- 7.4



Agilent Seahorse XF Media, Buffer and Supplement Products

Part No	Product Name	Real-Time ATP Rate Assay	Cell Mito Stress Test	Glycolytic Rate Assay	Glycolysis Stress Test	Cell Energy Phenotype Test	Mito Fuel Flex Test
103575-100	Seahorse XF DMEM Medium, pH 7.4	●	●	●	●	●	●
103576-100	Seahorse XF RPMI Medium, pH 7.4	✓	✓	✓	✓	✓	✓
103335-100	Seahorse XF Base Medium (without Phenol Red)	✓ ●	✓	✓ ●	✓	✓	✓
103336-100	Seahorse XF RPMI Medium (without Phenol Red)	✓ ●	✓	✓ ●	✓	✓	✓
102353-100	Seahorse XF Base Medium, 2 x 1 L						
103334-100	Seahorse XF Base Medium, 500 mL	●	✓	●	✓	✓	✓
103193-100	Seahorse XF Base Medium, 100 mL						

● Recommended

✓ Compatible

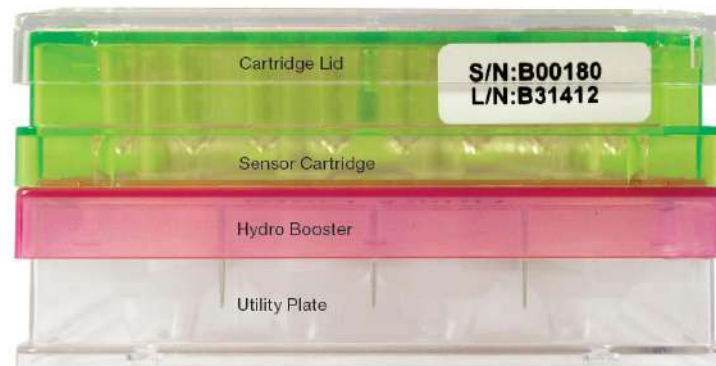
● Not Compatible

● Require addition of HEPES



Hydrate Cartridge

- Add **1000** mL Calibrant Solution
- Place in a non-CO₂ 37 °C incubator **OverNight**
- Keep cartridge humidified
- Remember to **REMOVE** the **Hydro Booster** and **Lid** before experiment



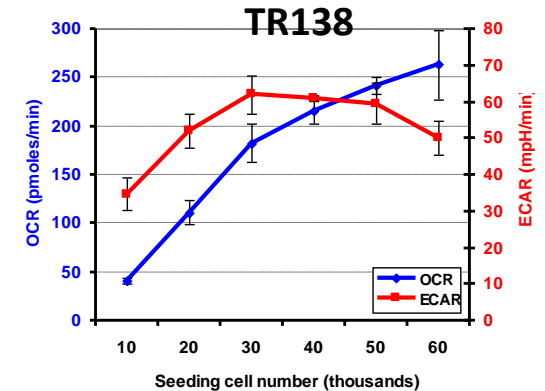
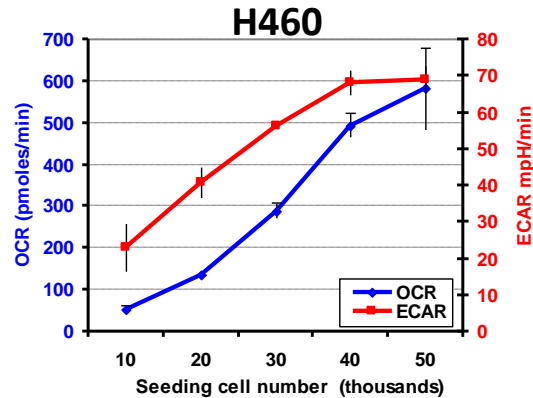
WARNING:
Remember to remove the Hydro Booster and Cartridge Lid prior to placing the Sensor Cartridge and Utility Plate in the XF²⁴ Analyzer.

Seed Cell

- Test with commercial 96well plate

Too **Less** cells -- Low signal

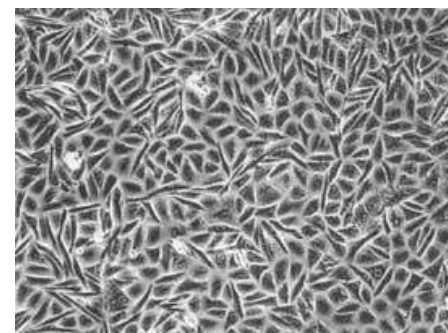
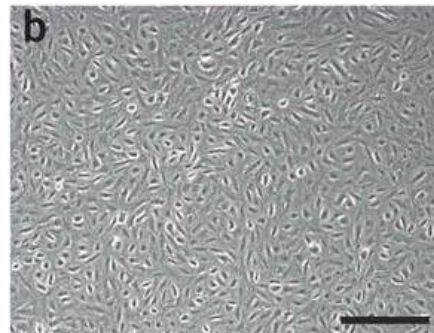
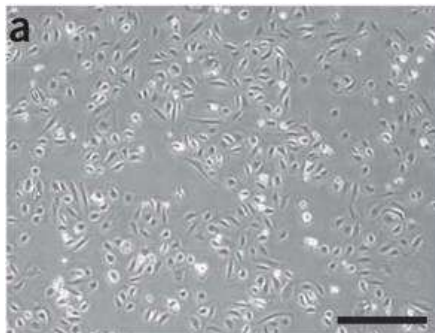
Too **Many** cells – Cell stress



- Seed cell to **Confluent** condition as assay the experiment

Optimal cell seeding numbers are typically between

10,000 – 80,000 cells per well



Cell Reference Database

Research Area

Cell Type

Cell Line

Analyzer

XF Analyzer Assay

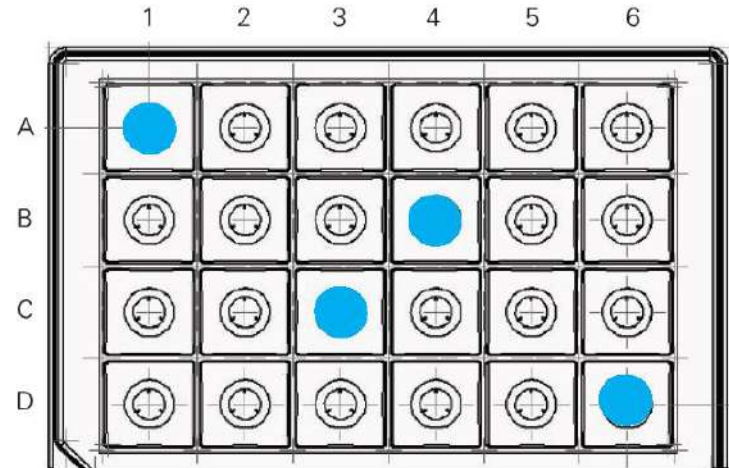
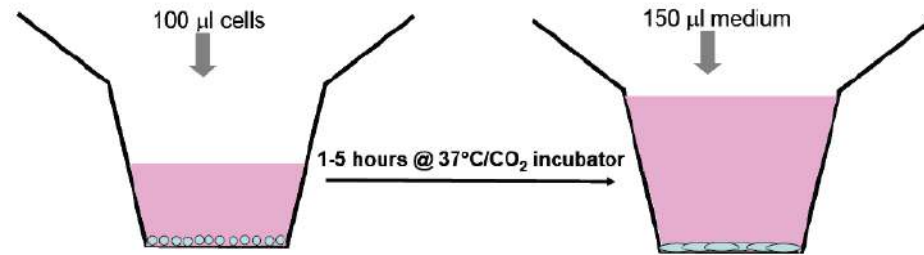
Plate Reader Assay

Author
Last Name, First Initial, eg:Yang Y

<http://www.seahorsebio.com/learning/cell-line.php>

2 Step Seeding

- Suspend the cells to desired conc. to seed in **100 μL** of growth medium.
- After cells have adhered, add **150 μL** of growth medium to each well.
- Do not seed cells in background correction wells (A1, B4, C3, D6)



Monitor growth and health of cells using a microscope before assay the experiment.



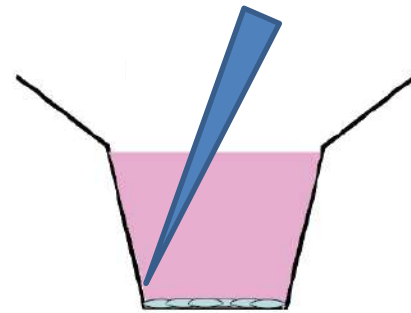
XF專用之細胞培養盤，擺放時缺角(左下角處)為前端，兩端均有用於辨識之條碼，圖中藍色位置為背景校正用，僅需要置入等量之培養基，不需種入細胞。



Prepare assay medium

- Warm assay medium to **37°C**
- Look at cells under the microscope to:
 - a. Confirm cell health, morphology, seeding uniformity and purity (no contamination).
 - b. Ensure cells are adhered, and no gaps are present.
 - c. Make sure no cells were plated in the background correction wells.

- Remove all culture medium from single well.
- Add **675 μL** of assay medium to well.
- Operate whole procedure per well.
- Also add 675 μL of assay medium to blank wells.

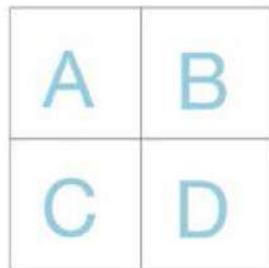


Prepare and load compound

- Dilute compound to correct conc. with **warmed assay medium**
- Load compound to correct position

Assay Medium	Injection	Concentration in Port
675 uL	75 uL	10X Compound A
750 uL	85 uL	10X Compound B
835 uL	95 uL	10X Compound C
930 uL	100 uL	10X Compound D
1030 uL		

Assay Medium	Injection	Concentration in Port
675 uL	75 uL	10X Compound A
750 uL	75 uL	11X Compound B
825 uL	75 uL	12X Compound C
900 uL	75 uL	13X Compound D
975 uL		



XF Dilution Calculation

FINAL Conc. (藥物最終濃度) x 10 (稀釋倍數) x VOL TO PREPARE (需要製備體積)

= STOCK (藥物保存濃度) x VOL STOCK SOLUTION (從Stock取多少體積的藥物)

範例：配製Oligomycin

1 uM x 10 x 1600 uL = 500 uM x VOL STOCK SOLUTION

VOL STOCK SOLUTION = 16000 / 500 = 32 uL

取32 uL stock oligomycin 到 1568 uL 上機用培養基

Design and Load Protocol



- 3 Steps complete assay design.

The screenshot displays the Wave software interface for designing an assay. The main window is titled "New Design" and has a navigation bar with "Group Definitions", "Instrument Protocol", and "Review and Run". Below this is a toolbar with icons for "Measure", "Injection", and "Custom". The "Total Time: 01:56:00" is shown in the top right corner.

The interface is divided into four vertical panels:

- Initialization:** Contains two steps: "Calibrate" (checked) and "Equilibrate" (checked). The "Calibrate" step includes a table for 4 Measurement Cycles.
- Basal:** Shows a table for 4 Measurement Cycles.
- Injection 1:** Shows "Select Ports" (A, B, C, D) with A and B selected, and a checked "Measure after Injection" option. Below is a table for 3 Measurement Cycles.
- Injection 2:** Shows "Select Ports" (A, B, C, D) with A and B selected, and a checked "Measure after Injection" option. Below is a table for 3 Measurement Cycles.
- Injection 3:** Shows "Select Ports" (A, B, C, D) with C and D selected, and a checked "Measure after Injection" option. Below is a table for 3 Measurement Cycles.

Each panel has a "Group Summary" section at the bottom. The time for each panel is displayed at the bottom: 00:32:00 for Basal, and 00:24:00 for each of the three injection steps.

Mix	Wait	Meas.
03:00	02:00	03:00

Mix	Wait	Meas.
03:00	02:00	03:00

Mix	Wait	Meas.
03:00	02:00	03:00

Mix	Wait	Meas.
03:00	02:00	03:00

Run assay

- Put on the plate with right direction.
- Barcode toward the front



FCCP Titeration Test

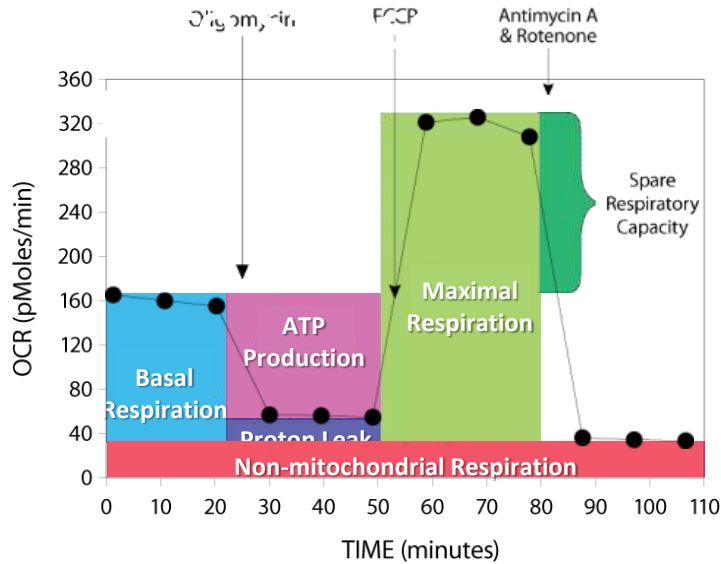
	1	2	3	4	5	6
A	Black circle on grid	Blue circle	Blue circle	Blue circle	Blue circle	Blue circle
B	Blue circle	Blue circle	Blue circle	Black circle on grid	Blue circle	Blue circle
C	Green circle	Green circle	Black circle on grid	Green circle	Green circle	Green circle
D	Green circle	Green circle	Green circle	Green circle	Green circle	Black circle on grid

- One Cell Line
- ✓ CTL
- ✓ Exp
- Two Cell Line
- ✓ CTL
- ✓ CTL

	1	2	3	4	5	6
A	Black circle on grid	Blue circle	Green circle	Purple circle	Yellow circle	Red circle
B	Blue circle	Green circle	Purple circle	Black circle on grid	Yellow circle	Red circle
C	Cyan circle	Magenta circle	Black circle on grid	Lime green circle	Brown circle	Pink circle
D	Cyan circle	Magenta circle	Lime green circle	Brown circle	Pink circle	Black circle on grid

- Normal Cell Line
FCCP: 4,2,1, 0.5 & 0.25 μ M
- Cancer Cell Line
FCCP: 2,1, 0.5, 0.25 & 0.125 μ M

FCCP Titeration Test



XF^e Mito Stress Test

