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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Planning Your Assay
1
• Obtain materials
• Reconstitute kits
• Review media requirements
Day Prior to Assay
2
• Hydrate cartridge
• Seed cells
Day of Assay
3
Prepare and run assay
• Prepare assay medium
• Wash cells
• Prepare assay compounds
• Load cartridge with compounds
• Design/load instrument protocol
• Run assay
After Your Assay
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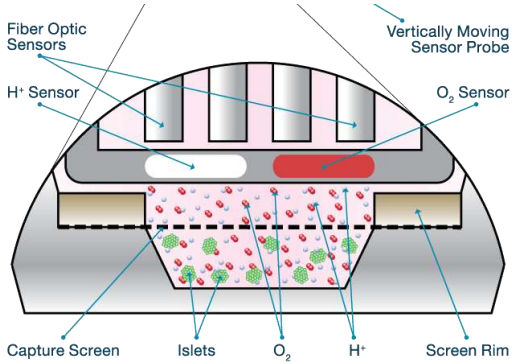
Data interpretation
After Your Assay
5
Next experiments

Consumables

XF24 FluxPaks

Islet FluxPaks



High Stability

High Reproducibility

Kits

Mito Stress Test Kit

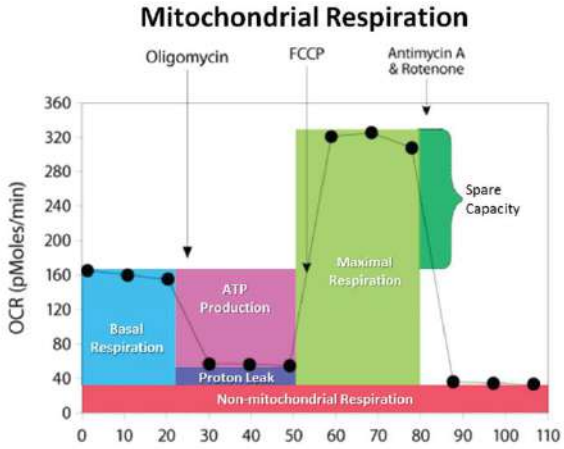
- ✓***Oligomycin***
- ✓***FCCP***
- ✓***Rotenone & Antimycin A***

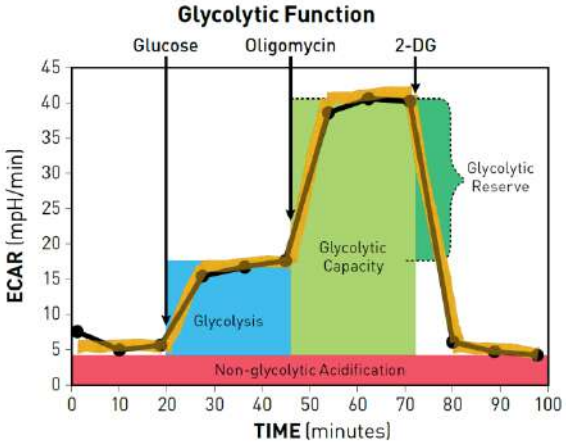
Glycolysis Stress Test Kit

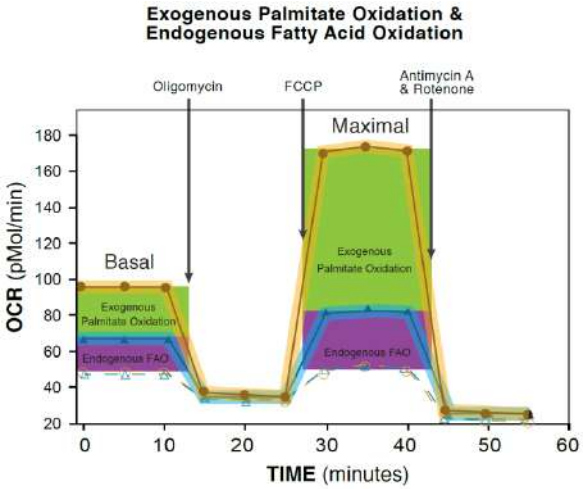
- ✓***Glucose***
- ✓***Oligomycin***
- ✓***2-DG***

Palmitate-BSA FAO Substrate

- ✓***BSA***
- ✓***Palmitate-BSA***

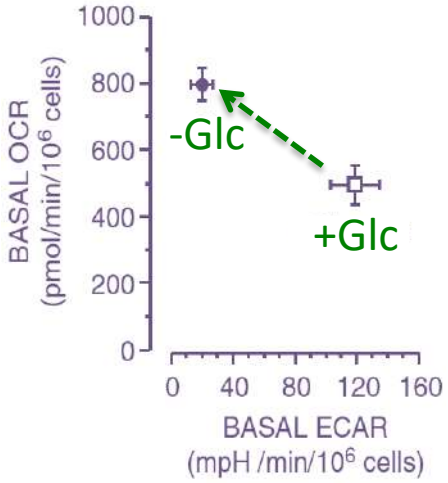






Media

- **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 103334-100 103193-100	Seahorse XF Base Medium, 2 x 1 L Seahorse XF Base Medium, 500 mL Seahorse XF Base Medium, 100 mL	●	✓	●	✓	✓	✓

● Recommended
✓ Compatible
● Not Compatible
● Require addition of HEPES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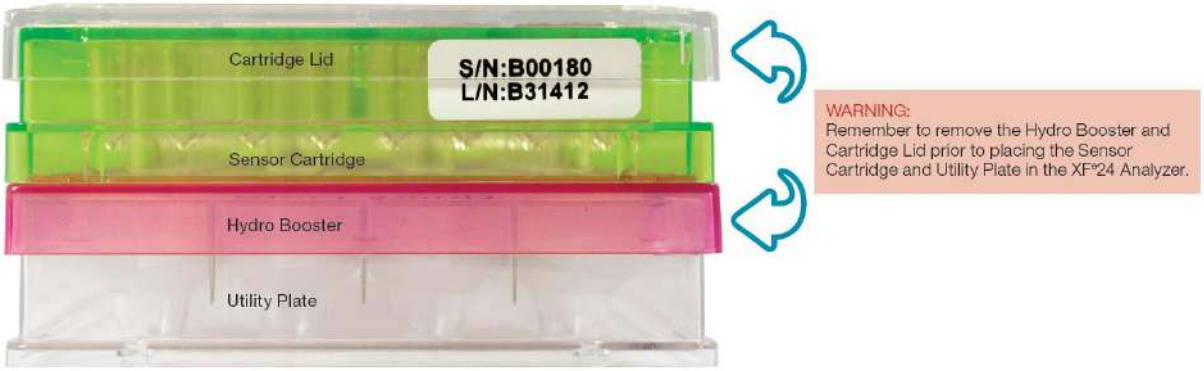
Hydrate Catridge

- Add **1000** mL Calibrant Solution
- Place in a non-CO_2 37 ˚C incubator
  OverNight

- Keep catridge humidified
- Remember to **REMOVE** the
  Hydro Booster and **Lid** before experiment

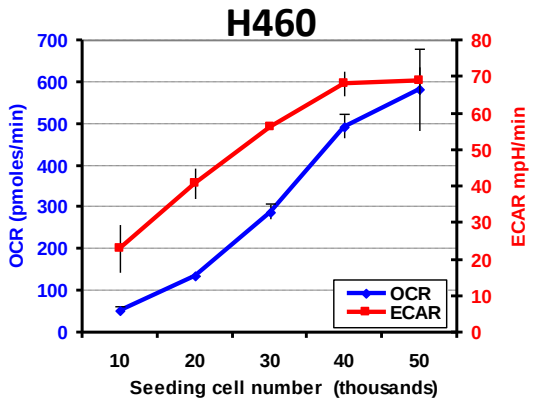


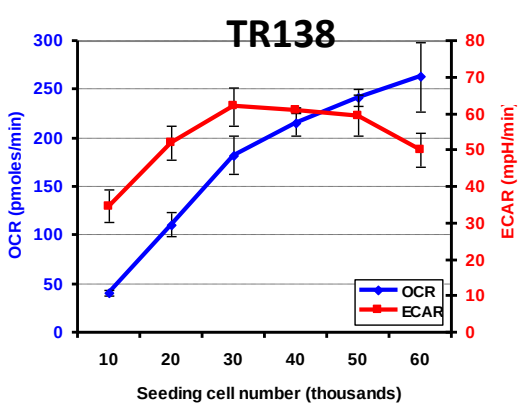
Seed Cell

- **Test with commercial 96well plate**

  Too **Less** cells -- Low signal

  Too **Many** cells – Cell stress

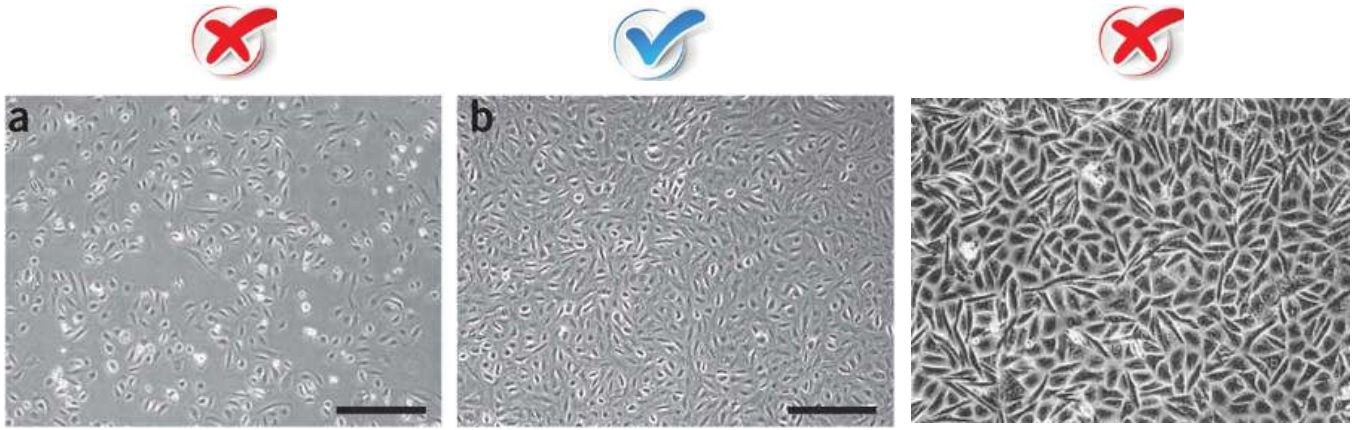




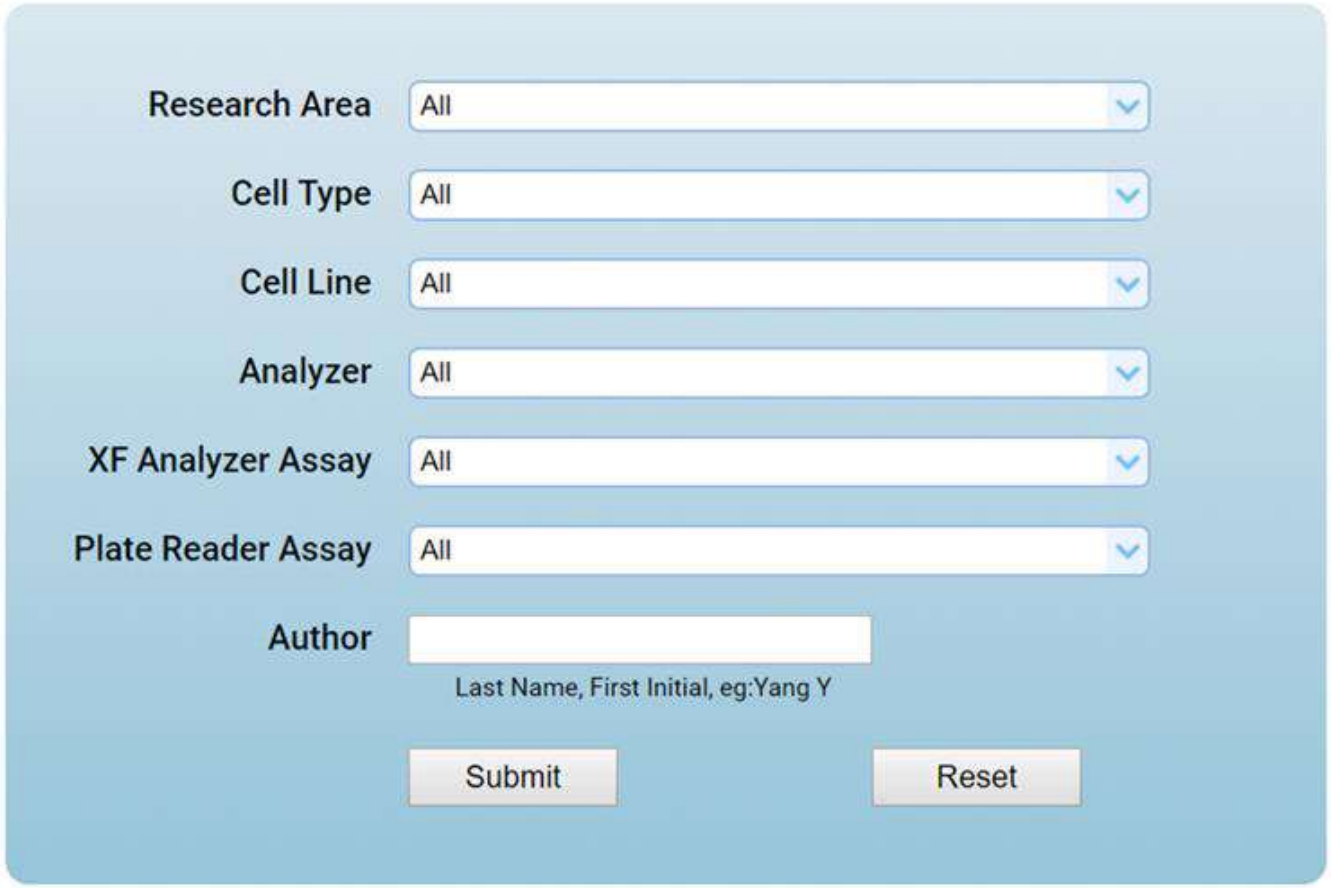
- **Seed cell to Confluent condition as assay the experiment**

  Optimal cell seeding numbers are typically between

  10,000 – 80,000 cells per well



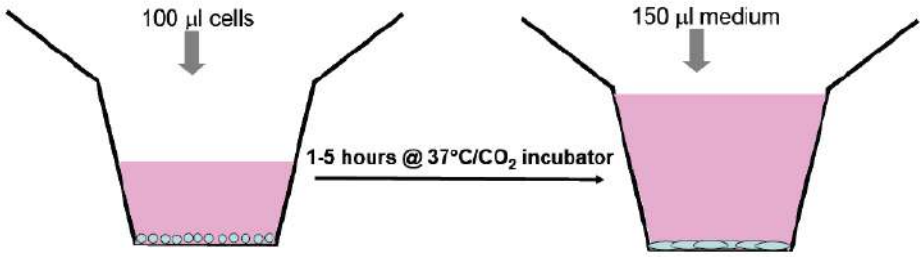
Cell Reference Database



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

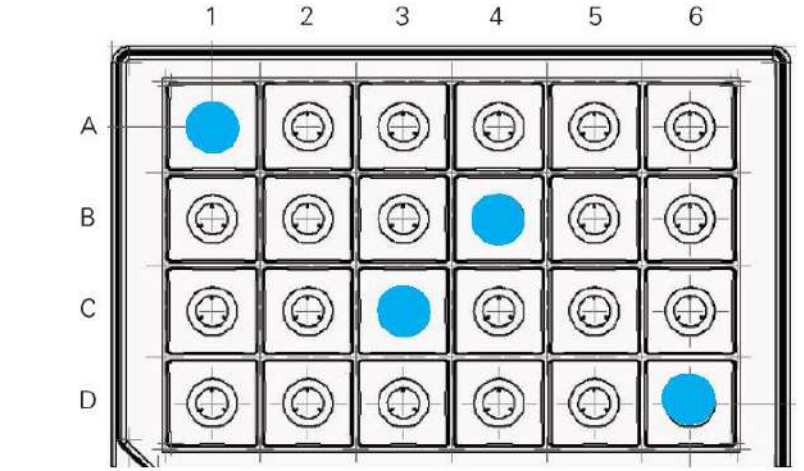
2 Step Seeding

- **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。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Prepare assay medium

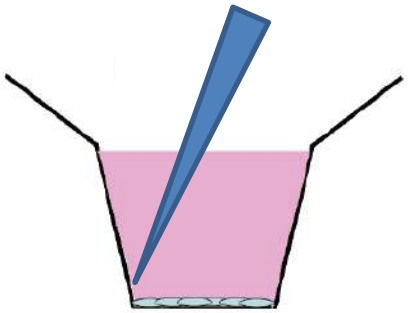
- Warm assay medium to **37**℃
- Look at cells under the microscope to:

  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 uL of assay medium to blank wells.

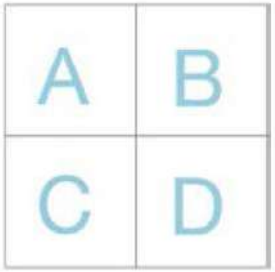


Prepare and load compound

- 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

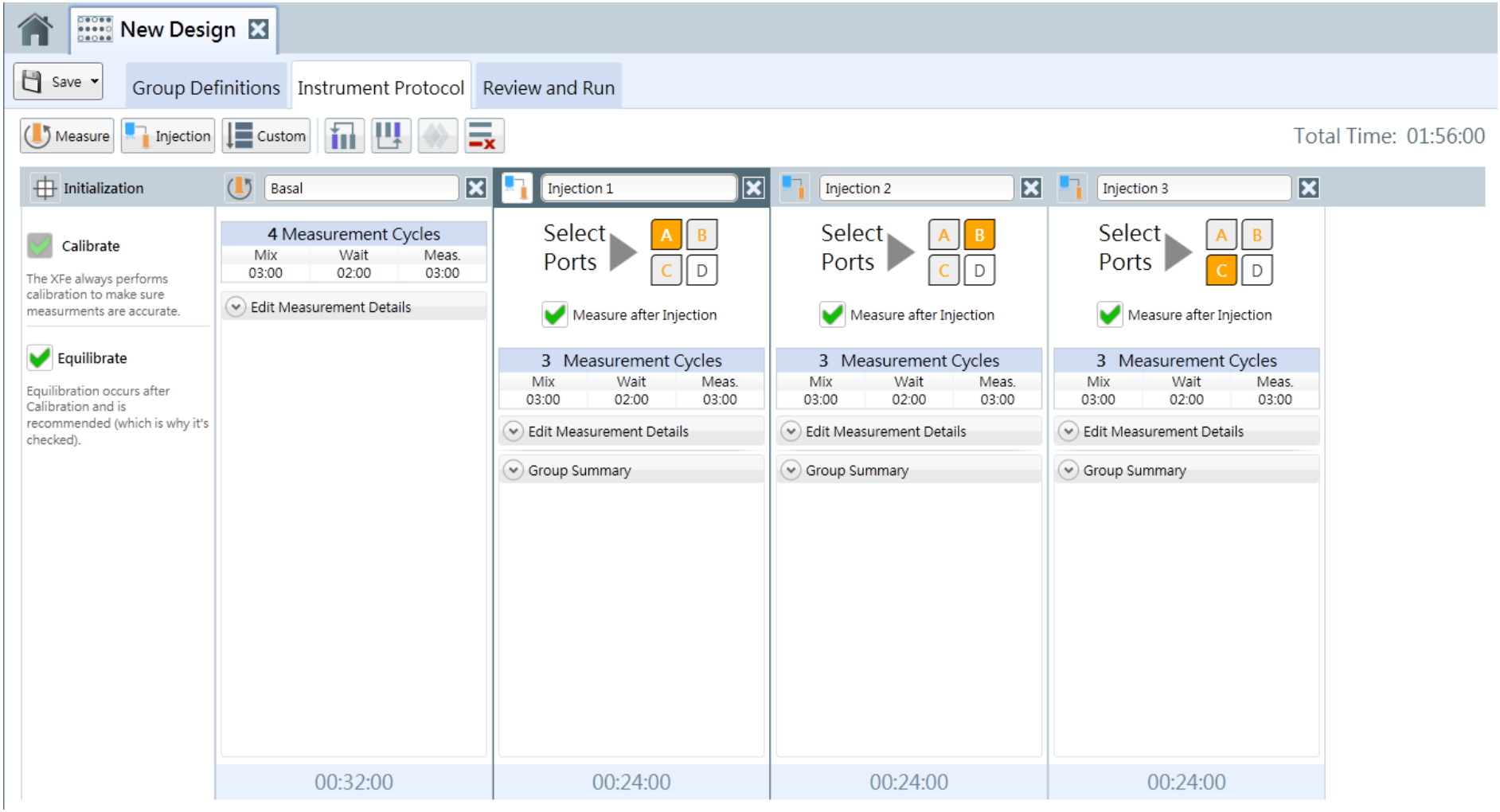
1 uM x 10 x 1600 uL = 500 uM x **VOL STOCK SOLUTION**

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

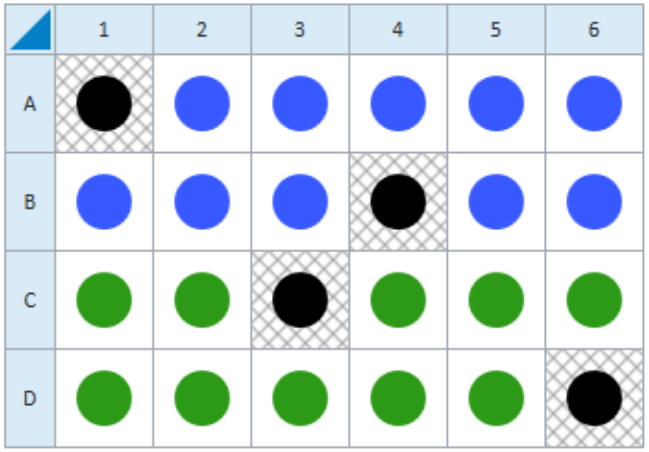
- **3 Steps complete assay design.**



Run assay

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

FCCP Titeration Test



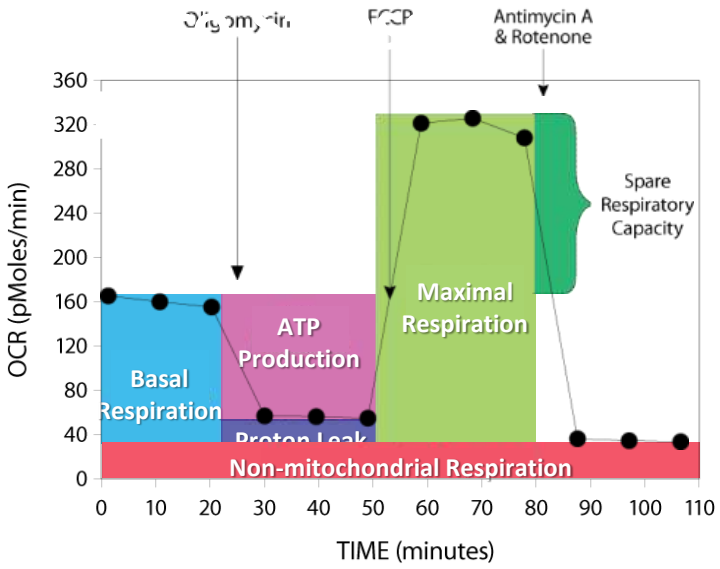
- One Cell Line
- ✓ CTL
- ✓ Exp
- Two Cell Line
- ✓ CTL
- ✓ CTL

- Normal Cell Line

  FCCP: 4,2,1, 0.5 & 0.25 uM
- Cancer Cell Line

  FCCP: 2,1, 0.5, 0.25 & 0.125 uM

FCCP Titeration Test



XF^{e} Mito Stress Test