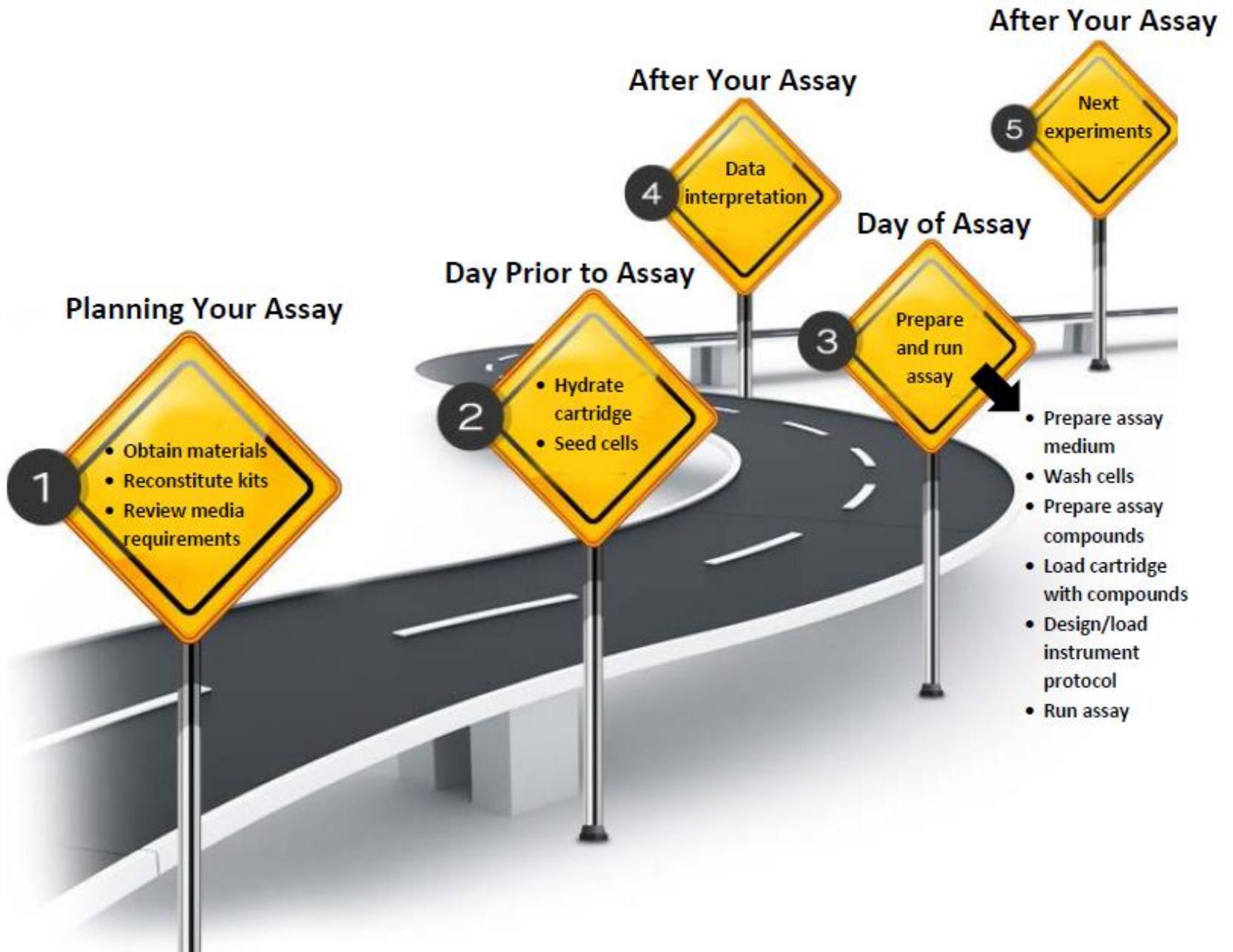


XF Flex Operating Training

Product Manager
Jack Chou





Consumables



XF^e24 FluxPaks

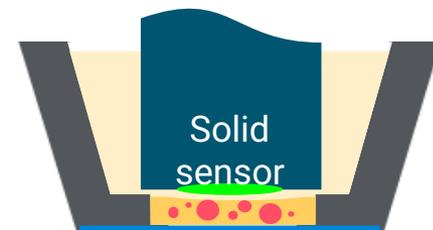
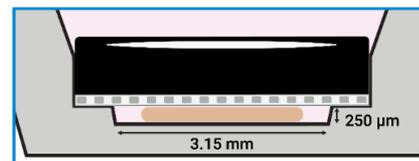
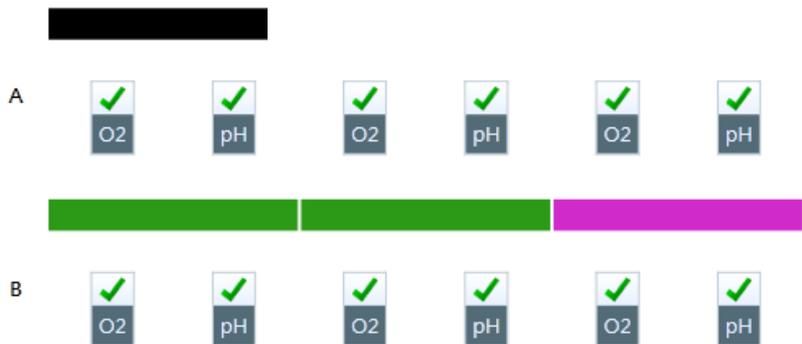


3D Capture MPLT-L



3D Capture Mplt-S

1



Flex Organoid Mplt

High Stability
High Reproducibility

Kits



Mito Stress Test Kit

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



Glycolysis Stress Test Kit

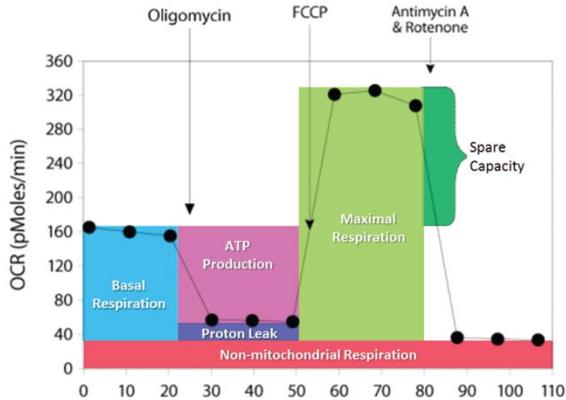
- ✓ *Glucose*
- ✓ *Oligomycin*
- ✓ *2-DG*



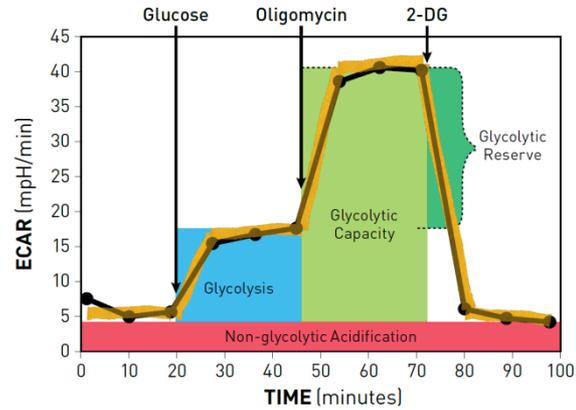
Palmitate-BSA FAO Substrate

- ✓ *BSA*
- ✓ *Palmitate-BSA*

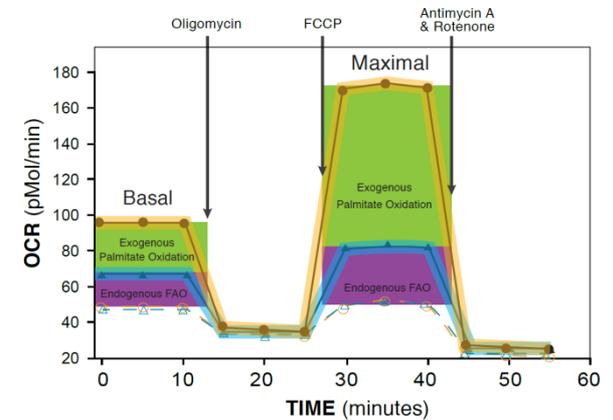
Mitochondrial Respiration



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

L-Alanyl-L-Glutamine (Stable Glutamine) (200 mM)



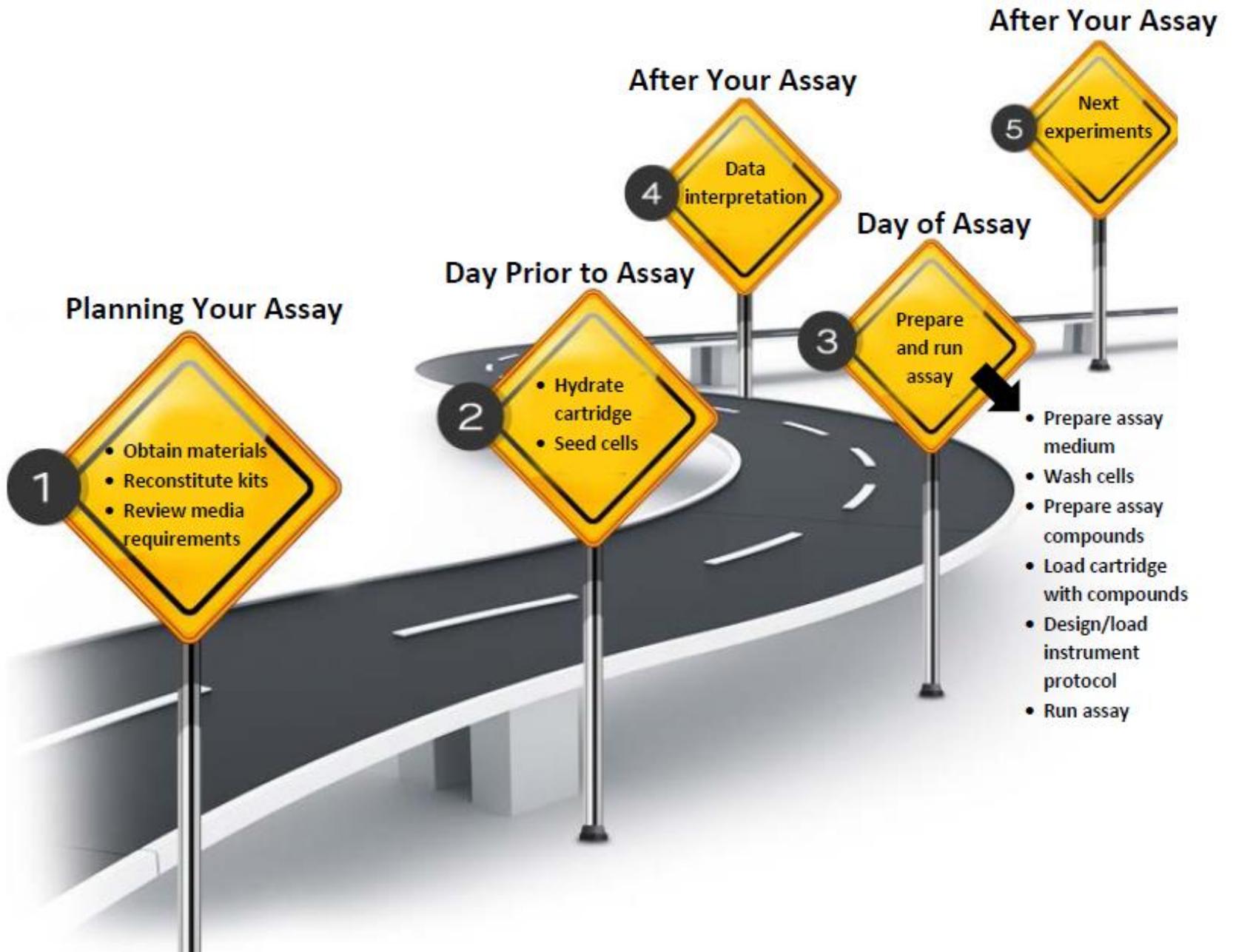
Stable alternative to L-glutamine

Minimizes toxic ammonia build-up

SKU: 03-022-1B

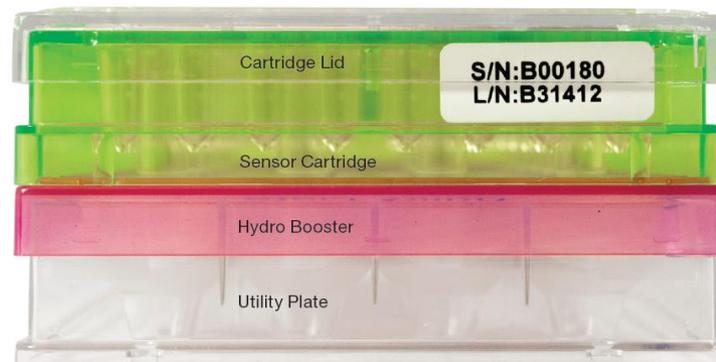
Part Number	Description	Quantity
103680-100	Seahorse XF DMEM assay medium pack: includes XF DMEM medium, pH 7.4, 500 mL (103575-100), XF 1.0 M Glucose Solution, 50 mL (103577-100), XF 100 mM Pyruvate Solution, 50 mL (103578-100), and XF 200 mM Glutamine Solution, 50 mL (103579-100).	
103681-100	Seahorse XF RPMI assay medium pack: includes XF RPMI medium, pH 7.4, 500 mL (103575-100), XF 1.0 M Glucose Solution, 50 mL (103577-100), XF 100 mM Pyruvate Solution, 50 mL (103578-100), and XF 200 mM Glutamine Solution, 50 mL (103579-100)	
103575-100	Seahorse XF DMEM Medium, pH 7.4	500 mL
103576-100	Seahorse XF RPMI Medium, pH 7.4	500 mL





Hydrate Cartridge

- Add **1** mL Calibrant Solution each well
 - Place in a non-CO₂ 37 °C incubator
- OverNight**
- Keep cartridge humidified
 - Remember to **REMOVE** the **Hydro Booster** and **Lid** before experiment



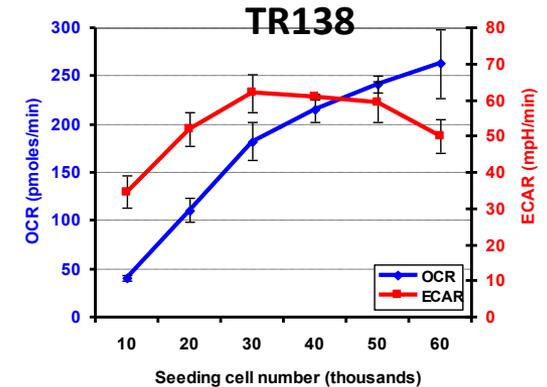
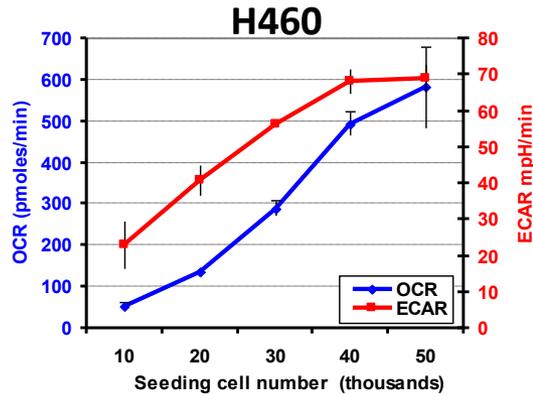
WARNING:
Remember to remove the Hydro Booster and Cartridge Lid prior to placing the Sensor Cartridge and Utility Plate in the XF*24 Analyzer.

Seed Cell

- Test with commercial 96well plate

Too **Less** cells -- Low signal

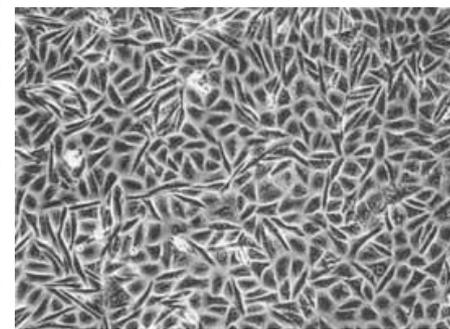
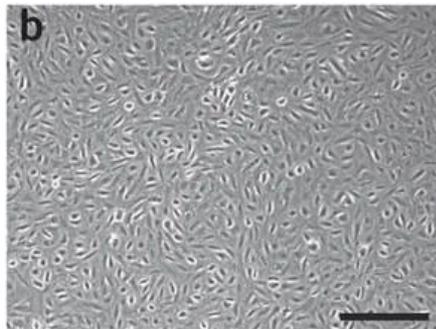
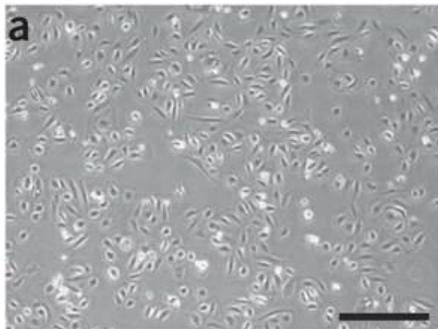
Too **Many** cells – Cell stress



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

Optimal cell seeding numbers are typically between

10,000 – 80,000 cells per well



Cell Type and Cell Line

Home > Search

PRODUCTS SOLUTIONS TRAINING & EVENTS SERVICES SUPPORT RESOURCES ORDER CENTER

0

— Cell Type

- Adenocarcinoma (2)
- Adipocytes (154)
- Adipose Tissue (60)
- Adrenocortical Carcinoma (3)
- Alveolar macrophages (2)

See more ...

— Cell Line

- 1205Lu (2)
- 143B (11)
- 143B 206 Rho+ (1)
- 143B TK(-) (4)
- 16HBE140 (1)

See more ...

+ Analysis Platform

- Not Specified (203)
- Review Article (53)

Agilent Cell Analysis Publication Database

The Agilent Cell Analysis Publication Database provides an easy way to search scientific publications that reference and/or cite Agilent products. Search publications by research area, cell type, cell line, instrument, assay, or author. The resulting publications can be reviewed with links to the abstract, in the Quick View, or be exported in MS Excel format and reviewed offline at a later time. (Click on the Export button to compile the results)

To be enabled for e-commerce, you must first register. Agilent will then confirm your registration and inform you when your enablement is complete and you can begin transacting.

[Download Results](#)

 Publication

Voluntary exercise delays progressive deterioration of markers of metabolism and behavior in a mouse model of Parkinson's disease [Read Abstract](#)

Journal: Brain Res / Publication Date: October 1, 2019 / Author: Lai JH, et al.

Quick View ^ Supporting Products v

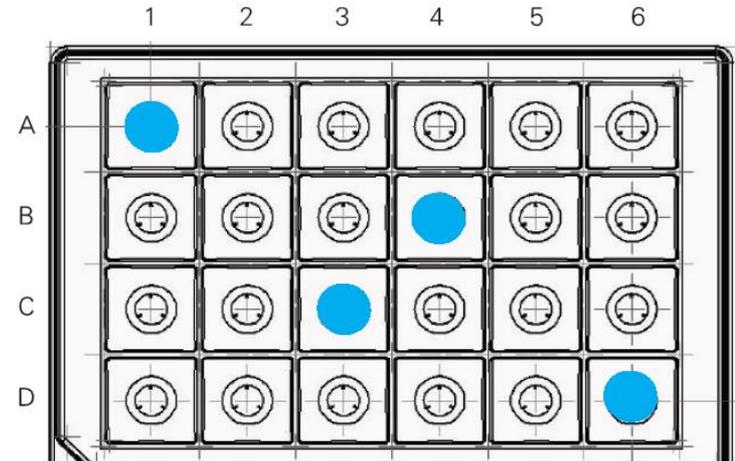
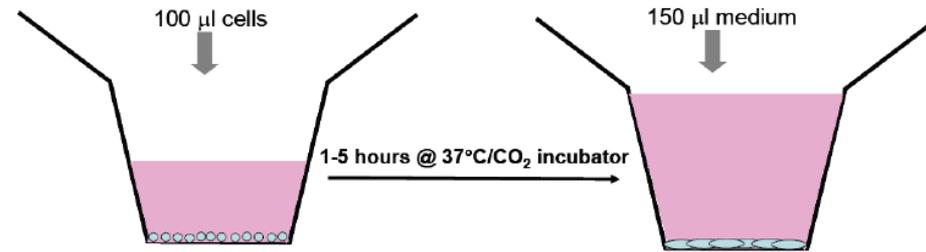
Authors	Lai JH, Chen KY, Wu JC, Olson L, Brené S, Huang CZ, Chen YH, Kang SJ, Ma KH, Hoffer BJ, Hsieh TH, Chiang YH
Journal	Brain Res
Publication Date	October 1, 2019
Research Area	Neurobiology Research
Cell Line	Nigrostriatal tissue
Cell Type	Nigrostriatal tissue
Assay	Basal Metabolic Assay
Species	Mouse
Cell Seeding Density	1.5x1.5x0.5mm ³ pieces/well
Plate Coating	Not Specified

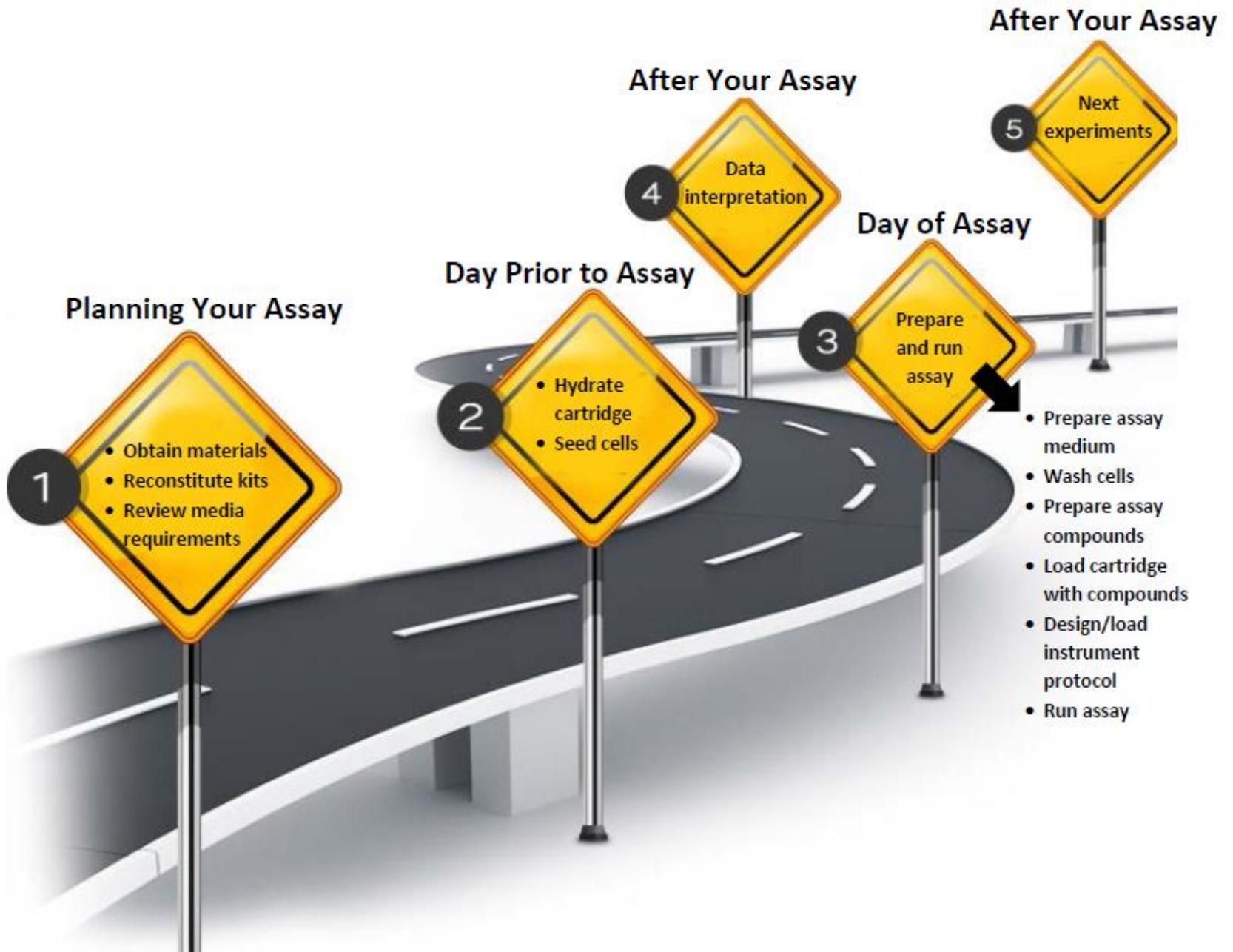
<https://www.agilent.com/search/?N=4294836537>

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.

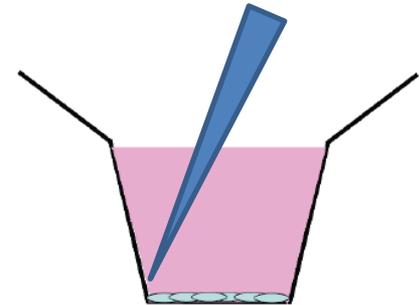




Prepare assay medium

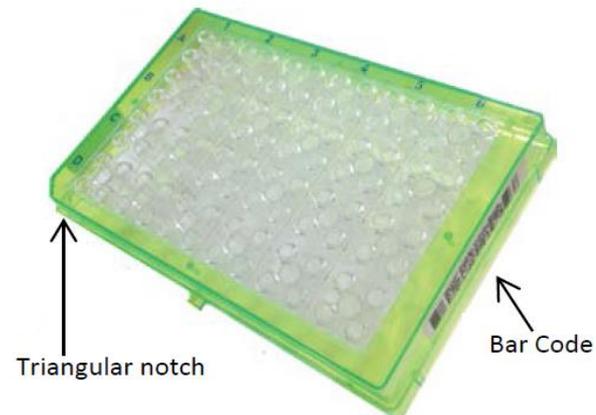
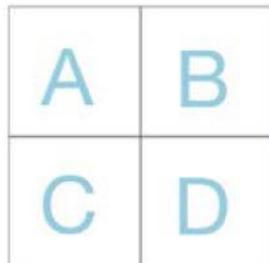
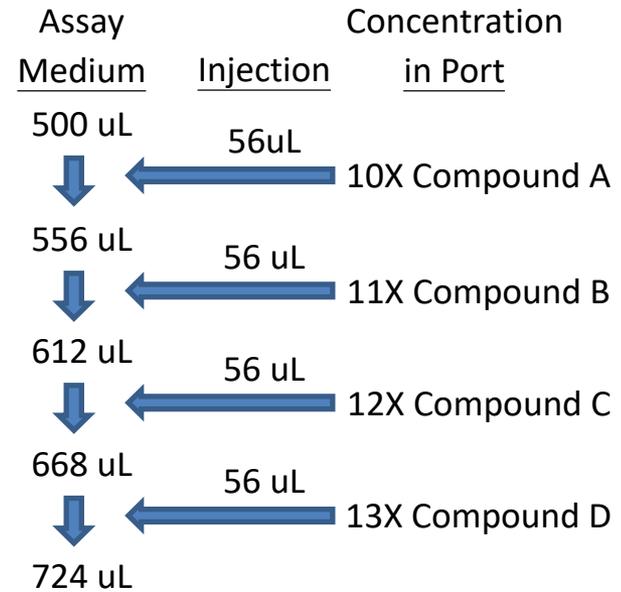
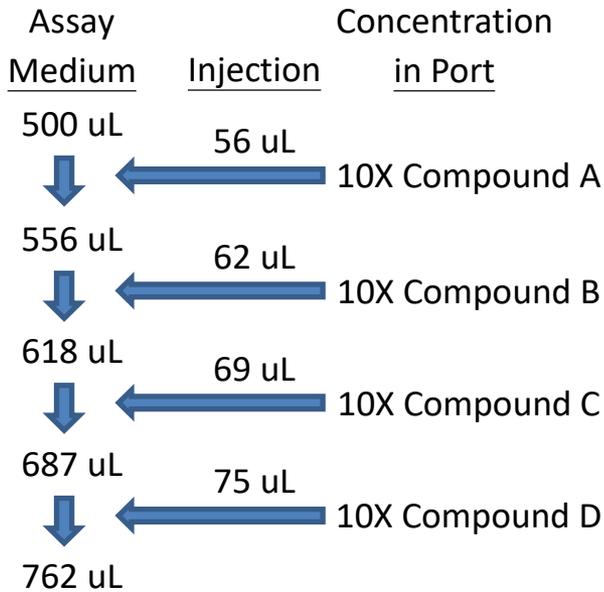
- Warm assay medium to **37°C**
- See cells under the microscope to confirm that...
 - a. Cell health, morphology, seeding uniformity and no contamination.
 - b. Cells are adhered well and spread averagely.

- Remove all culture medium from single well.
- Add **500 µL** of assay medium to well.
- Operate whole procedure per well.
- Also add 500 uL of assay medium to blank wells.



Prepare and load compound

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



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 contains several tabs: "Group Definitions", "Instrument Protocol", and "Review and Run". The "Instrument Protocol" tab is active, showing a sequence of steps: "Measure", "Injection", and "Custom". The "Total Time: 01:56:00" is displayed in the top right corner.

The assay design is organized into four columns, each representing a different step:

- Initialization:** Includes "Calibrate" (checked) and "Equilibrate" (checked). The "Calibrate" step is described as: "The XFe always performs calibration to make sure measurements are accurate." The "Equilibrate" step is described as: "Equilibration occurs after Calibration and is recommended (which is why it's checked)."
- Basal:** Shows "4 Measurement Cycles" with a table:

Mix	Wait	Meas.
03:00	02:00	03:00
- Injection 1:** Shows "3 Measurement Cycles" with a table:

Mix	Wait	Meas.
03:00	02:00	03:00
- Injection 2:** Shows "3 Measurement Cycles" with a table:

Mix	Wait	Meas.
03:00	02:00	03:00
- Injection 3:** Shows "3 Measurement Cycles" with a table:

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

Each injection step includes a "Select Ports" section with a 2x2 grid of buttons labeled A, B, C, and D. In the "Injection 1" step, buttons A and B are highlighted in orange. In the "Injection 2" step, buttons A and B are highlighted in orange. In the "Injection 3" step, buttons C and D are highlighted in orange. Each injection step also includes a "Measure after Injection" checkbox, which is checked.

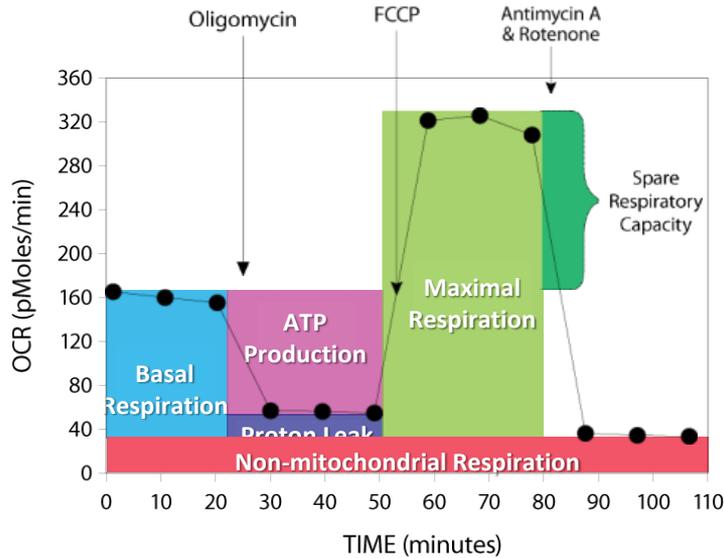
At the bottom of each column, the time for that step is displayed: 00:32:00 for Basal, and 00:24:00 for each of the three injection steps.

Run assay

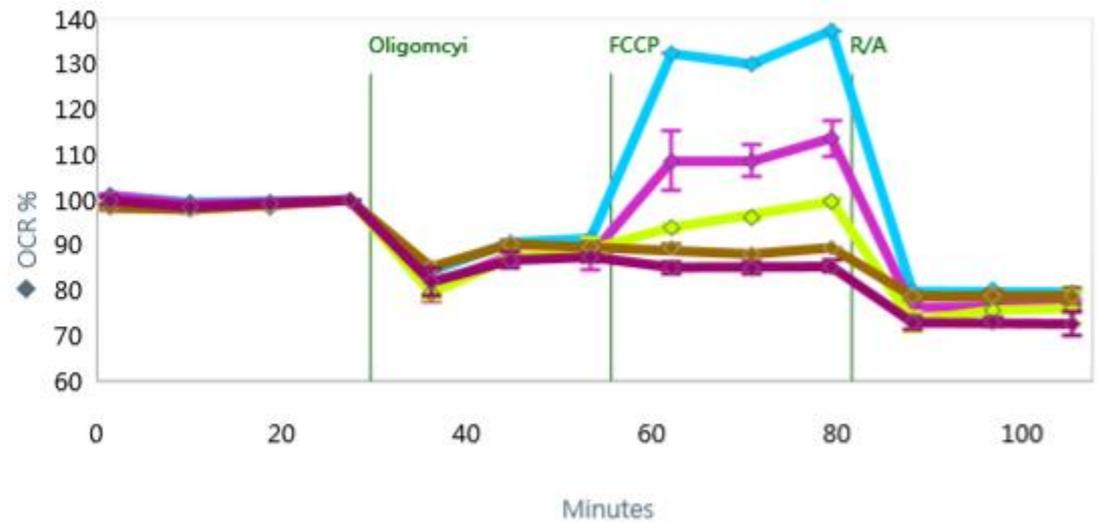
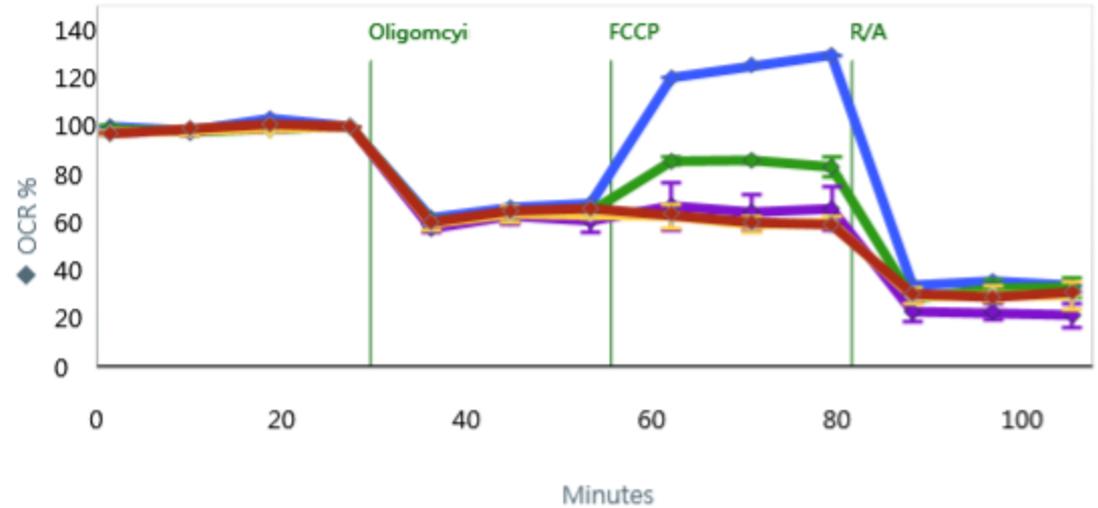
- Put on the plate with right direction.
- Barcode toward the front



FCCP Titeration Test

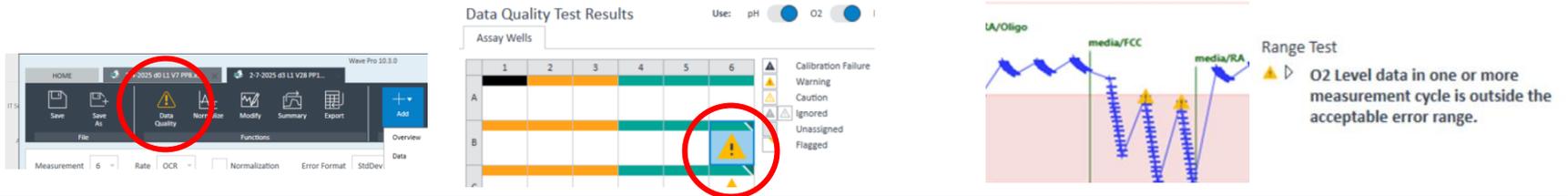


XF^e Mito Stress Test



Seahorse Analytics and Wave Pro

- Automated data QC



Test applied when selecting "Data Quality" icon:

	XF V7	XF V28	XF Flex 3D Capture plate
Background test <ul style="list-style-type: none"> Identifies unexpected data changes (sudden or drifting) – only O₂ Identifies cells present in background wells 	✓	✓	✓
Level tests <ul style="list-style-type: none"> Data range checks Identifies sudden data changes 	✓	✓	✓
Rate test <ul style="list-style-type: none"> Lower Limit of Detection check 	✓	✓	–
Injection test (2 test sets) <ul style="list-style-type: none"> Identifies injection failures Identifies injection problems 	✓	✓	–
Temperature test <ul style="list-style-type: none"> Verifies if the instrument set point temperature matches the actual tray temperature during an assay 	✓	✓	✓
Calibration test (performed on all wells) <ul style="list-style-type: none"> Identifies which wells failed calibration: O₂ or pH or both 	✓	✓	✓

Agilent Seahorse Analytics Report Generator

(Any PC)



Wave Pro
(Windows only)



DATA ANALYSIS

The screenshot displays the Agilent Seahorse Analytics software interface. The top navigation bar includes Home, Files, Projects, and Resources. The main content area is titled "Add View" and features a sidebar menu on the left with categories like "Standard Views", "My Custom Views", and "Assay Kit Companion Views". Under "XF ATP Rate Assays", the "XF ATP Rate Assay" is selected. The main dashboard shows "XF ATP Rate Assay Widgets" with filters for "Oligo Injection" (1st) and "Induced Injection" (N/A). The widgets include:

- mitoATP Production Rate (Basal and Induced Rates)**: Line graph showing mitoATP Production Rate (pmol/min) over time.
- glycoATP Production Rate (Basal and Induced Rates)**: Line graph showing glycoATP Production Rate (pmol/min) over time.
- ATP Production Rate (Basal and Induced Rates)**: Line graph showing ATP Production Rate (pmol/min) over time.
- ATP Production Rate (Basal)**: Stacked bar chart showing glyco ATP Production Rate (red) and mito ATP Production Rate (blue) for three groups.
- ATP Production Rate (Induced)**: Stacked bar chart showing glyco ATP Production Rate (red) and mito ATP Production Rate (blue) for three groups.
- Energy Map (Basal)**: Scatter plot showing mitoATP Production Rate (pmol/min) vs glycoATP Production Rate (pmol/min).

A "Groups" panel on the right lists 12 groups with corresponding color swatches. The "Description" section at the bottom provides an overview: "This Companion View is designed to compare and rank Seahorse XF Real-Time ATP Rate Assay data for screening potent metabolic modulators."

<https://www.agilent.com/en/product/cell-analysis/real-time-cell-metabolic-analysis/xf-software/agilent-seahorse-analytics-787485>

Normalization

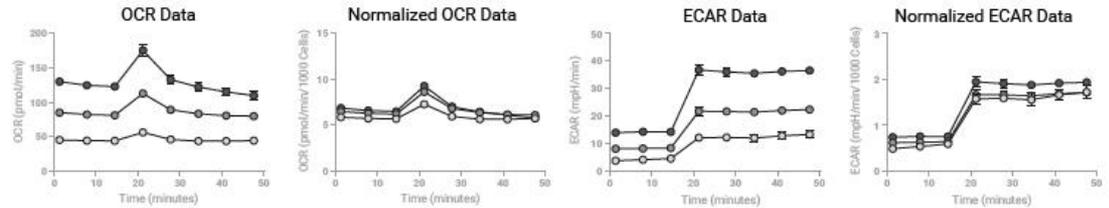
BioTek
Cytation 1/5

Seahorse XFe and
XF Pro Analyzers



Seahorse
Controller

- ✓ Standardized normalization method
- ✓ XF data interpretation made easier



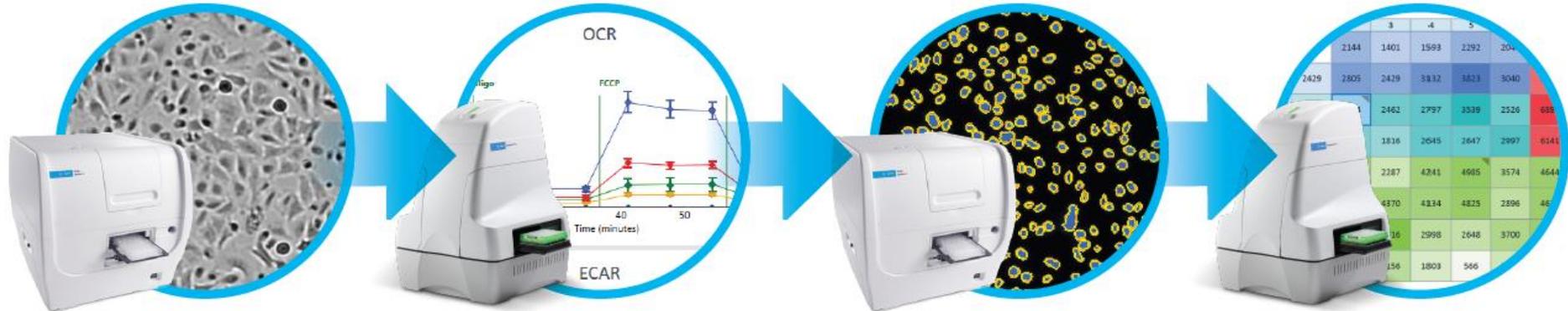
SKOV3 cells were plated at 10, 20, and 30K cells per well. Raw OCR and ECAR change with injection of oligomycin + FCCP.

Document

Analyze

Normalize

Interpret



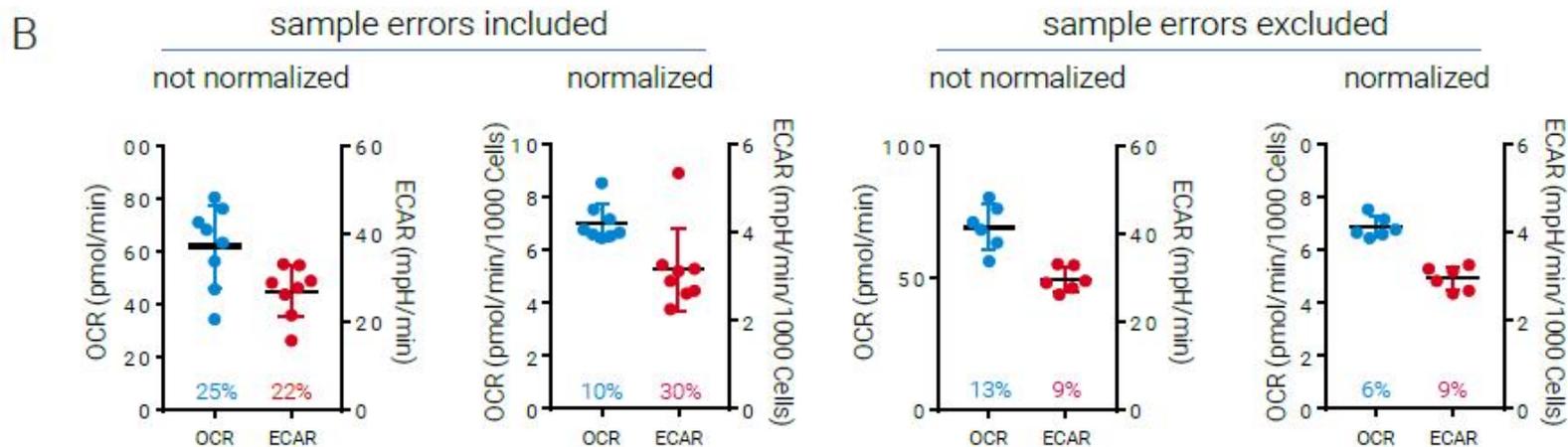
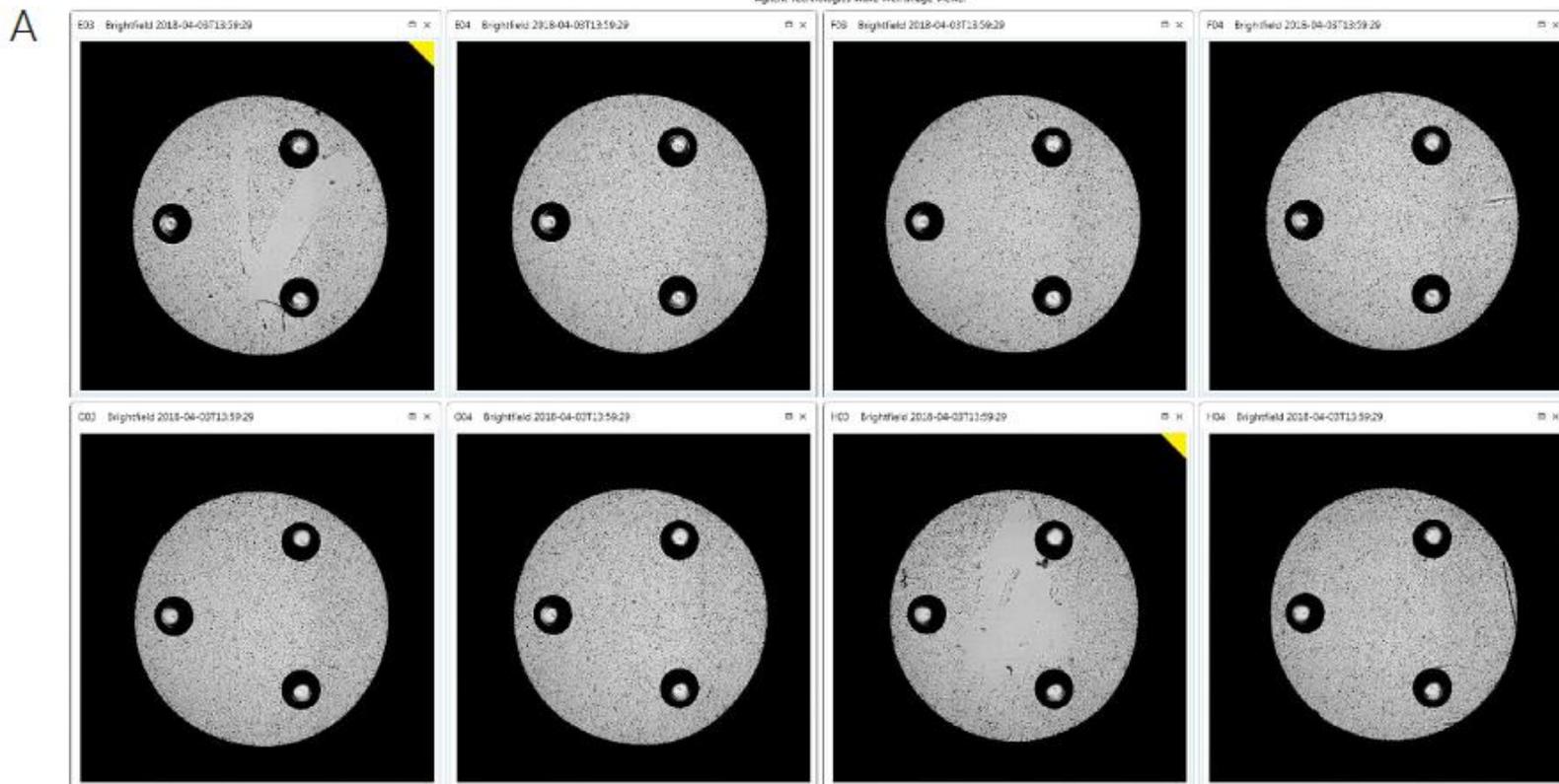
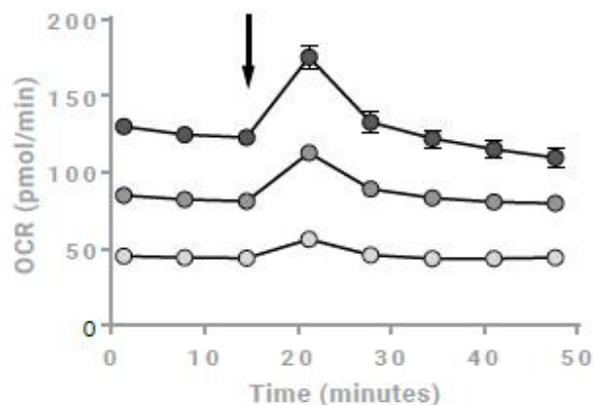


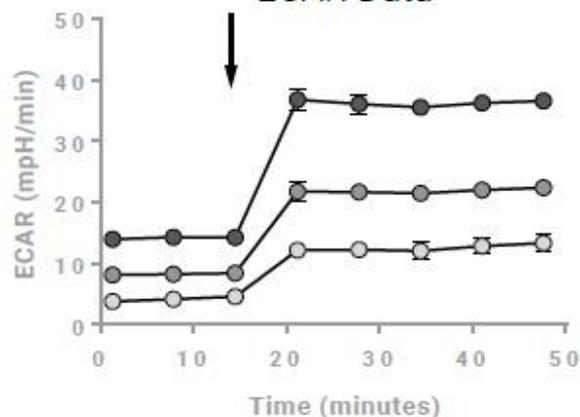
Figure 1. Error identification and documentation for XF analysis using brightfield images. A) Whole well brightfield images of A549 cells are compared by using the "well comparison" function in Wave software. Wells experiencing cell loss are marked by yellow flags on the upper right corner. B) Basal OCR and ECAR comparison before and after outlier exclusion. The % values on each graph are the corresponding %CVs.

A

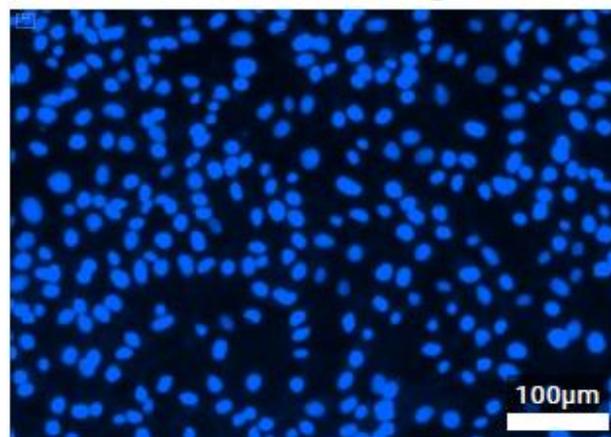
OCR Data



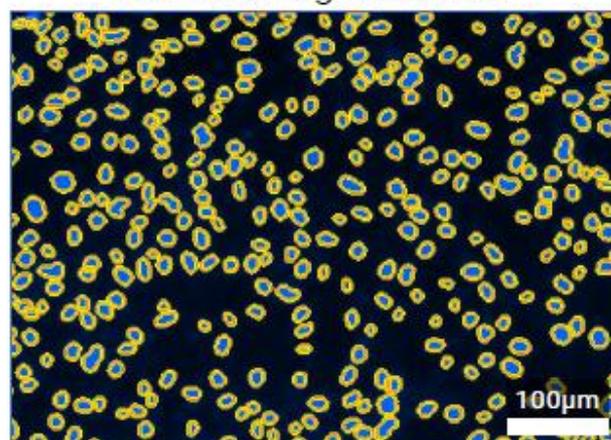
ECAR Data



B

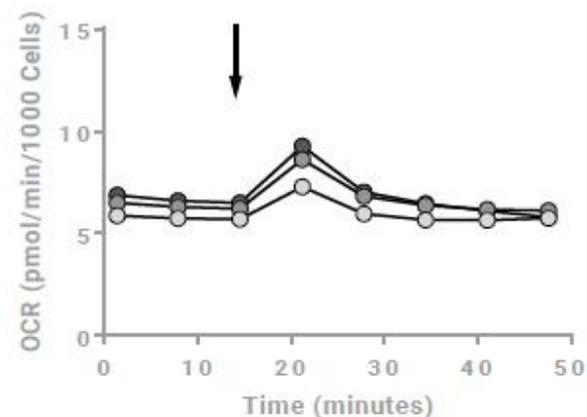
In situ Staining

Nuclear Segmentation



C

Normalized OCR Data



Normalized ECAR Data

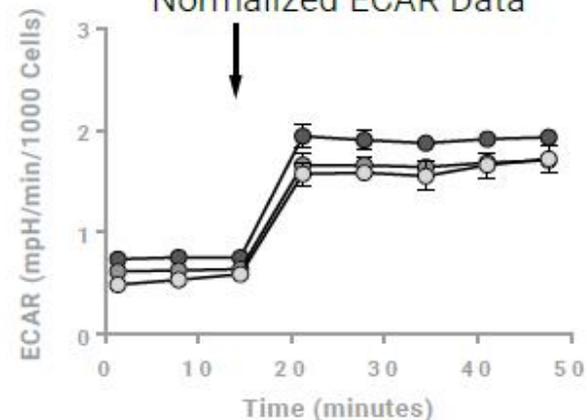
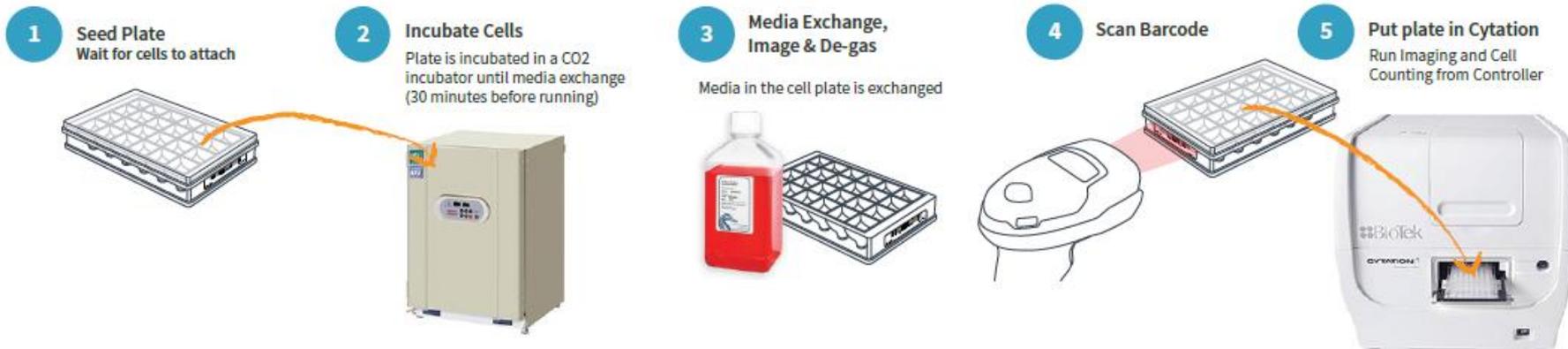


Figure 2. Example of XF data normalization using *in situ* nuclear staining and *in situ* cell counting. SKOV3 cells were plated at 1×10^4 , 2×10^4 , 3×10^4 cells per well, cultured 24 h, subject to the XF Cell Energy Phenotype Test and followed by image analysis. A) Raw OCR and ECAR change with injection (arrows) of oligomycin + FCCP ($1.0 \mu\text{M}$ and $0.5 \mu\text{M}$ final, respectively), including $20 \mu\text{M}$ Hoechst 33342 ($2 \mu\text{M}$ final). B) Representative images of nuclei fluorescently labeled by Hoechst 33342 (upper panel) and nuclei identified and outlined using the Cytation 1 (lower panel). C) OCR and ECAR normalized by *in situ* nuclear staining cell counts (Mean \pm SD, $n=4$).





6 Perform Brightfield Scan

Brightfield

Brightfield scans are most commonly used as an initial check immediately after seeding as well as post CO2 incubation. They can also be used post staining.

After scanning, the Cytation can provide non-CO2 incubation. A timer will let you know when outgassing is complete.

Standard Brightfield Scan



Live preview of images during scanning. User can flag wells. Image set is stored on the Controller for access by Wave. User can also export scans directly from the App to a graphic file (TIFF, JPG, etc)

Please note there may be more than 1 image set depending on cell type and growth duration.

7

Once scan is complete it can stay in the Cytation (Non CO2 incubator) to de-gas.

De-Gas timer 1 hour from start of scan

During downtime while degassing:

- Load injection ports on the cartridge, one with stain
- Place cartridge with utility plate (with calibrant), into XF and run calibration

Calibration 20 minutes

- When outgassing is done move the plate from the Cytation to the XF Analyzer



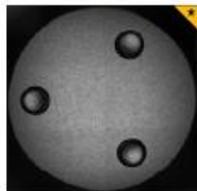
8 XF Run

Plate is placed in XF for Assay Run. Confirm design with staining injection. Run Assay.



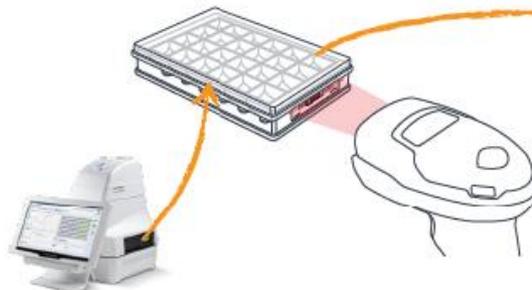
During XF Run:

- Review images in Imaging and Cell Counting App
- Flag bad wells



9 XF Run Complete

Remove stained plate from XF

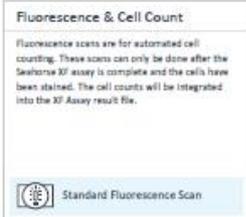


10 Scan Barcode

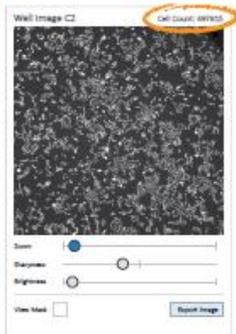
11 Put Plate in Cytation

12 Perform Fluorescence & Cell Count Scan

Run Imaging and Cell Count App.



13 Calculation of cell count



Processing of cell count should take about 20-30 minutes. Images and cell counts appear at the end of the scan. Cell Count masks are viewable. User can flag wells.

14 Scan Complete. Go to Wave

Imaging and Cell Count App prompts user to open Wave. User opens Assay within Wave. User selects Normalization screen.



Alternately, user can export scans directly from the Imaging and Cell Count App to a graphic (TIFF, JPG, etc) file.

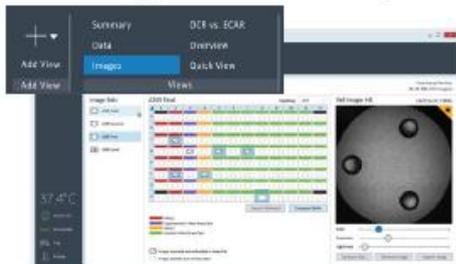
15 Edit Normalization Data

User imports cell counts. User can then view and edit cell counts imported from Cytation. Overwrite with their own data. Reimport data from Cell Counts.



16 Images View

User can add Images View from the finished assay ribbon.



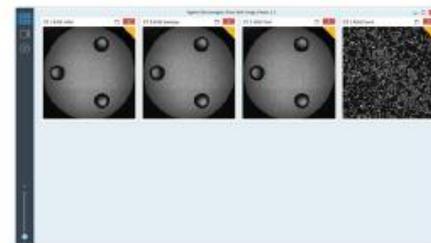
From the Image View user can see available image sets, select well images, compare them and embed them. Users can also view cell count data.

17a Comparing

User can compare multiple images from within a set and get up close on specific images and their data.

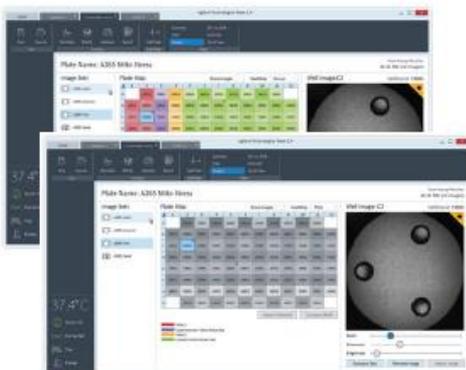


or Compare Sets to see what how one well compares across all sets.



17b Cell Counts

User can view cell counts and visualize comparisons with Heat Maps based on Groups or the entire plate.



17c Embedding

User can embed images into the Assay for future analysis in Wave Desktop.



18 Transfer ASYR to Desktop

Once images have been imported into an assay, the images are embedded into the ASYR file. The Assay can be taken to Wave Desktop for analysis with embedded images and cell counts.



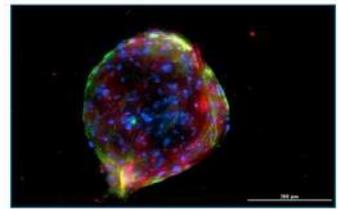
19 Manage

User can return to Imaging and Cell Counting App to manage images and sets in the image database.

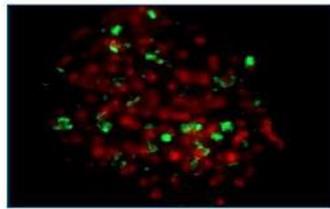
User can:

- view images
- rename sets/plates
- add/remove flags
- delete images/sets/plates
- export as full resolution TIFF

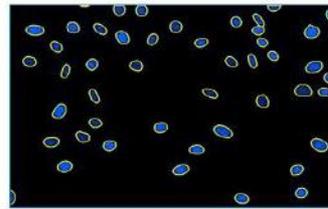
Cytation更多實驗應用



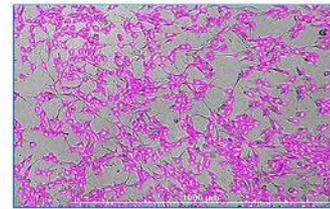
3D細胞培/類器官



細胞凋亡



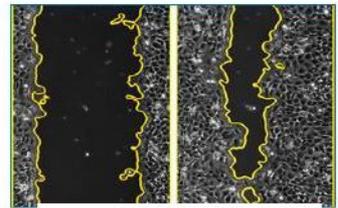
細胞計數



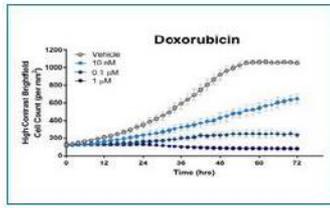
無標記細胞計數



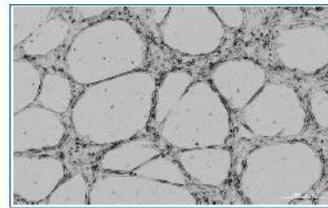
Elispot



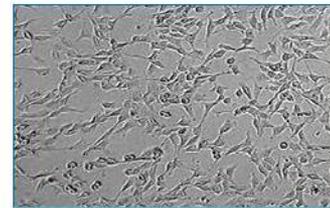
細胞劃痕/細胞遷移



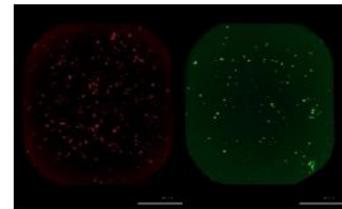
細胞增殖/活力/毒性



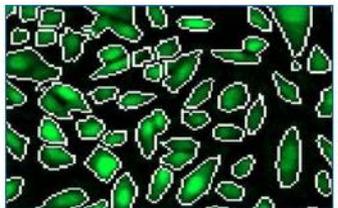
血管生成



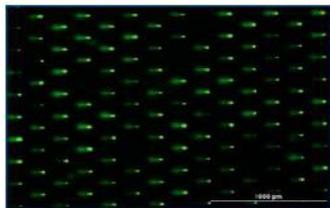
細胞覆蓋率



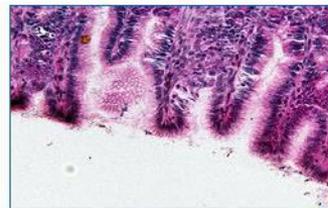
中和抗體檢測



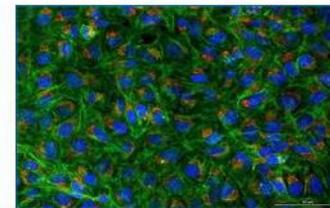
鈣離子檢測/快速動力學影像



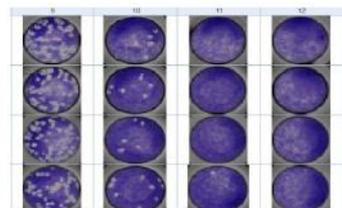
基因毒性/DNA損傷



組織學



細胞/組織免疫熒光



空斑檢測
Plaque Assay