XF Flex
Operating Training

Product Manager
Jack Chou

Agilent
Trusted Answers

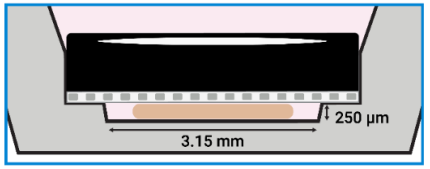

Planning Your Assay
1
• Obtain materials
• Reconstitute kits
• Review media requirements
Day Prior to Assay
2
• Hydrate cartridge
• Seed cells
Day of Assay
3
Prepare and run assay
• Prepare assay medium
• Wash cells
• Prepare assay compounds
• Load cartridge with compounds
• Design/load instrument protocol
• Run assay
After Your Assay
4
Data interpretation
After Your Assay
5
Next experiments

Consumables

XF^{e}24 FluxPaks

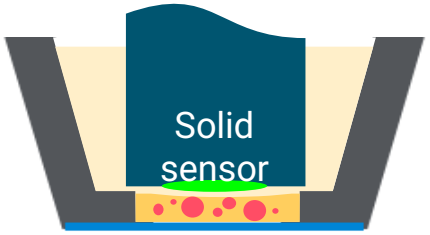
3D Capture MPLT-L

3D Capture Mplt-S



High Stability

High Reproducibility



Flex Organoid Mplt

Kits

Mito Stress Test Kit

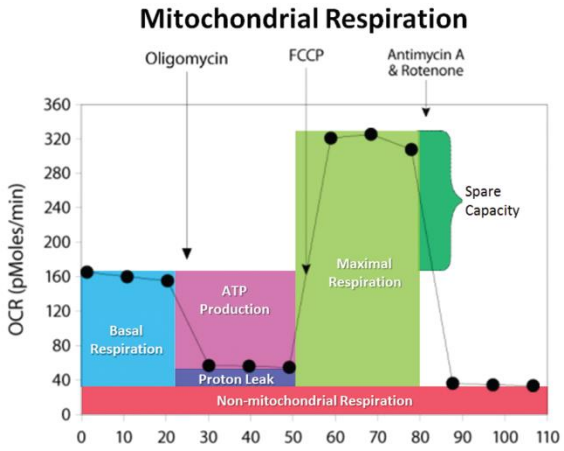
- ✓***Oligomycin***
- ✓***FCCP***
- ✓***Rotenone & Antimycin A***

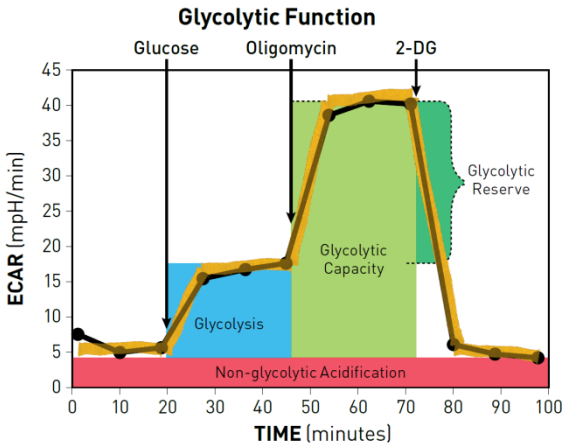
Glycolysis Stress Test Kit

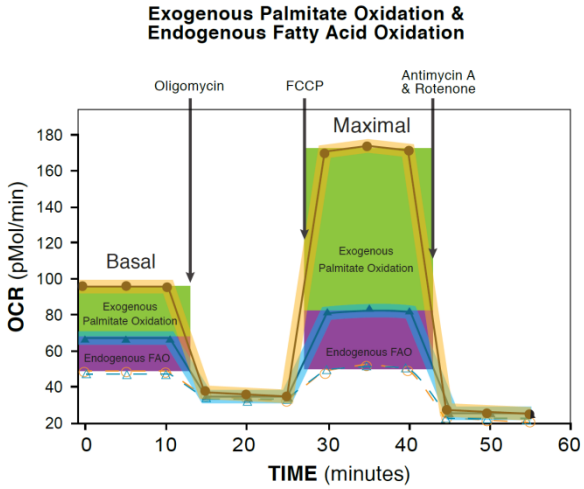
- ✓***Glucose***
- ✓***Oligomycin***
- ✓***2-DG***

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

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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Hydrate Catridge

- Add **1** mL Calibrant Solution each well
- Place in a non-CO_2 37 ˚C incubator
  OverNight

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

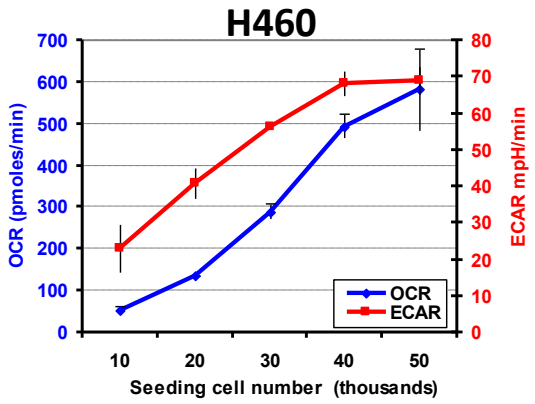
WARNING:
Remember to remove the Hydro Booster and Cartridge Lid prior to placing the Sensor Cartridge and Utility Plate in the XF^{e}24 Analyzer.

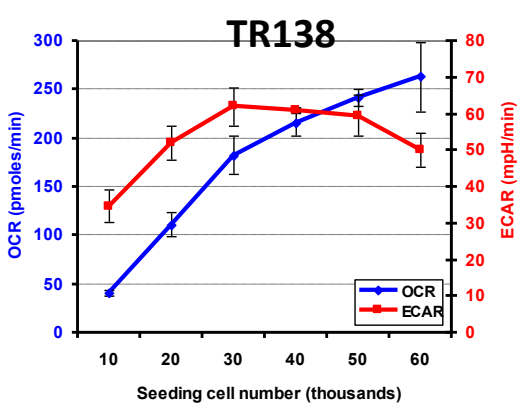
Seed Cell

- **Test with commercial 96well plate**

  Too **Less** cells -- Low signal

  Too **Many** cells – Cell stress

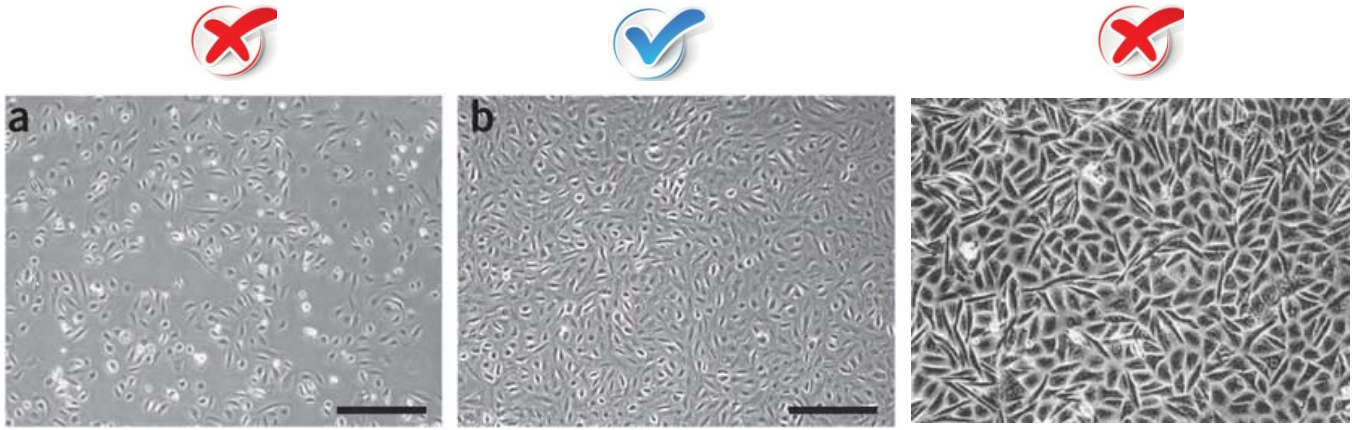




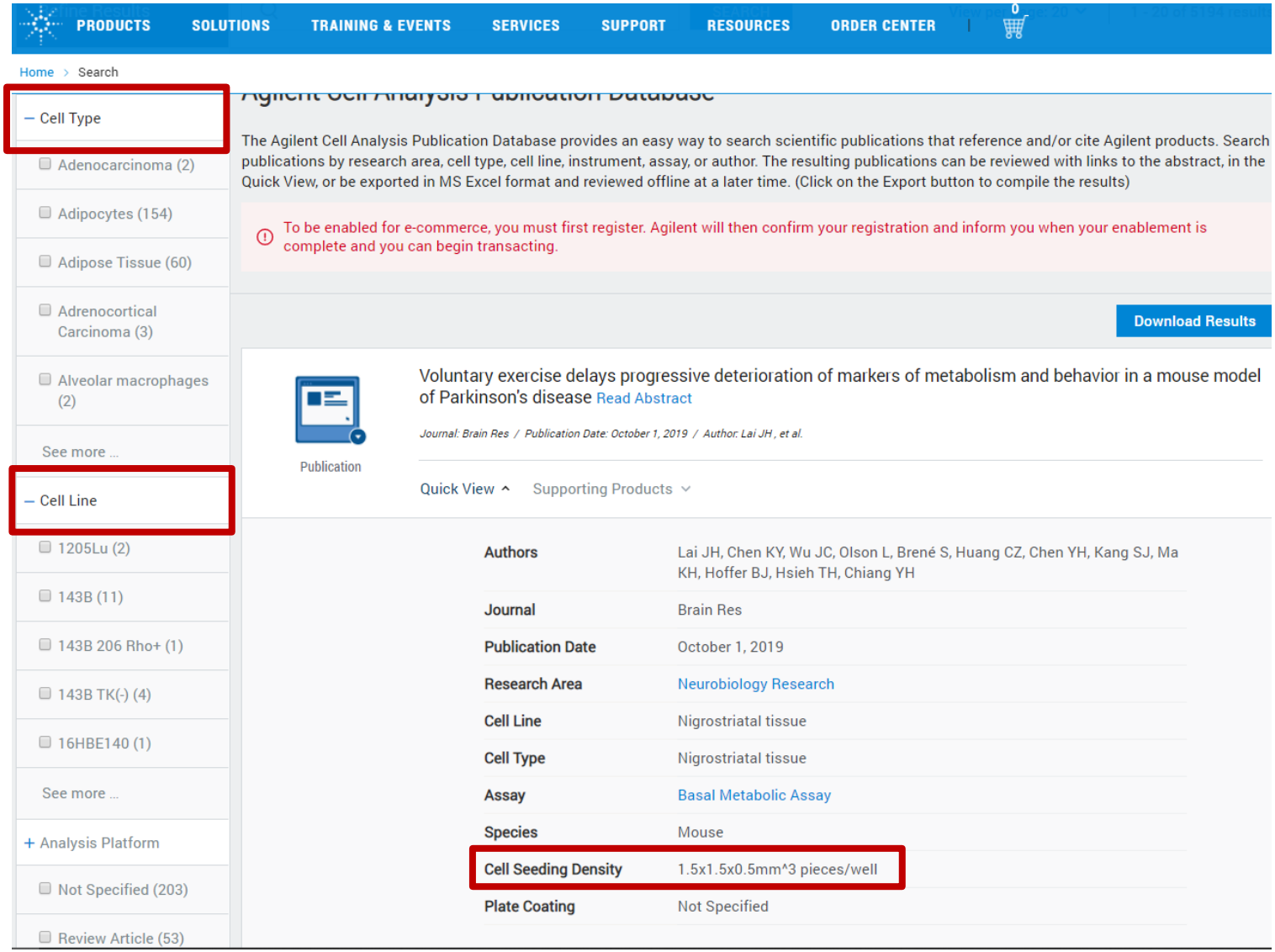
- **Seed cell to Confluent condition as assay the experiment**

  Optimal cell seeding numbers are typically between

  10,000 – 80,000 cells per well



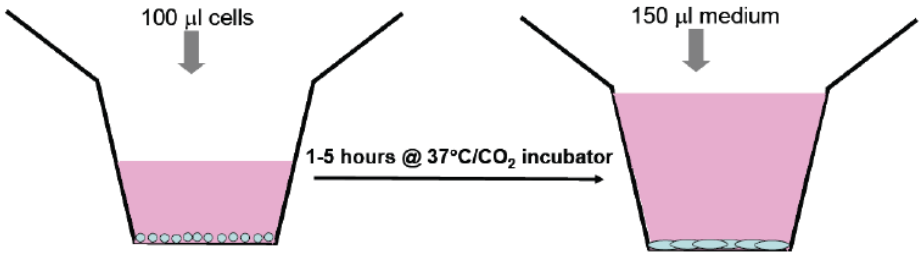
Cell Type and Cell Line



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

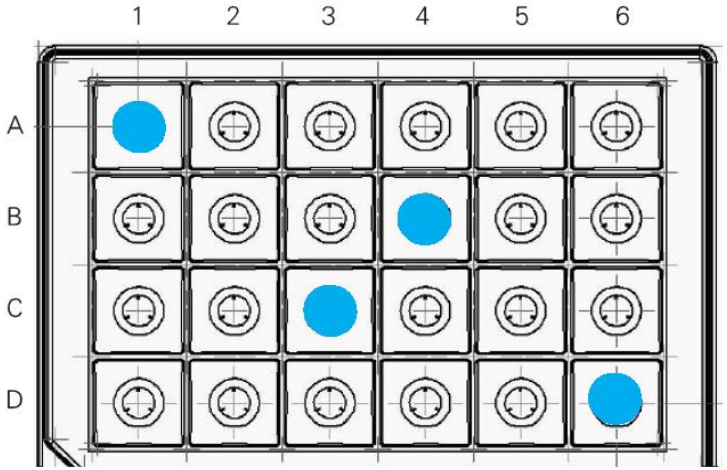
2 Step Seeding

- **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.

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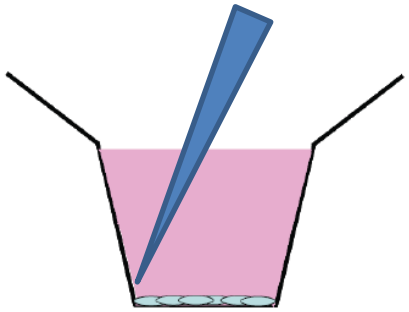
Prepare assay medium

- Warm assay medium to **37℃**
- 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.

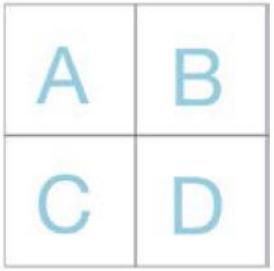


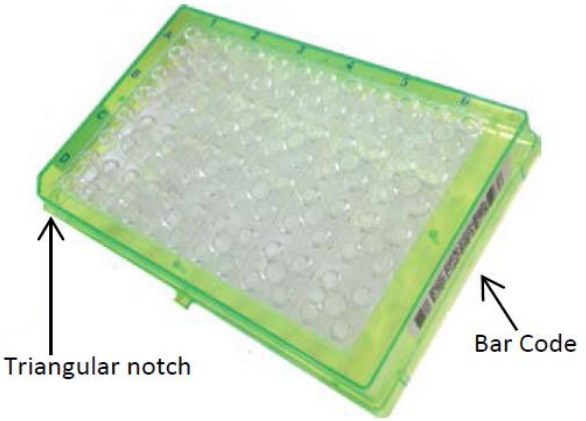
Prepare and load compound

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

Assay Medium	Injection	Concentration in Port
500 uL	56 uL	10X Compound A
556 uL	62 uL	10X Compound B
618 uL	69 uL	10X Compound C
687 uL	75 uL	10X Compound D
762 uL		

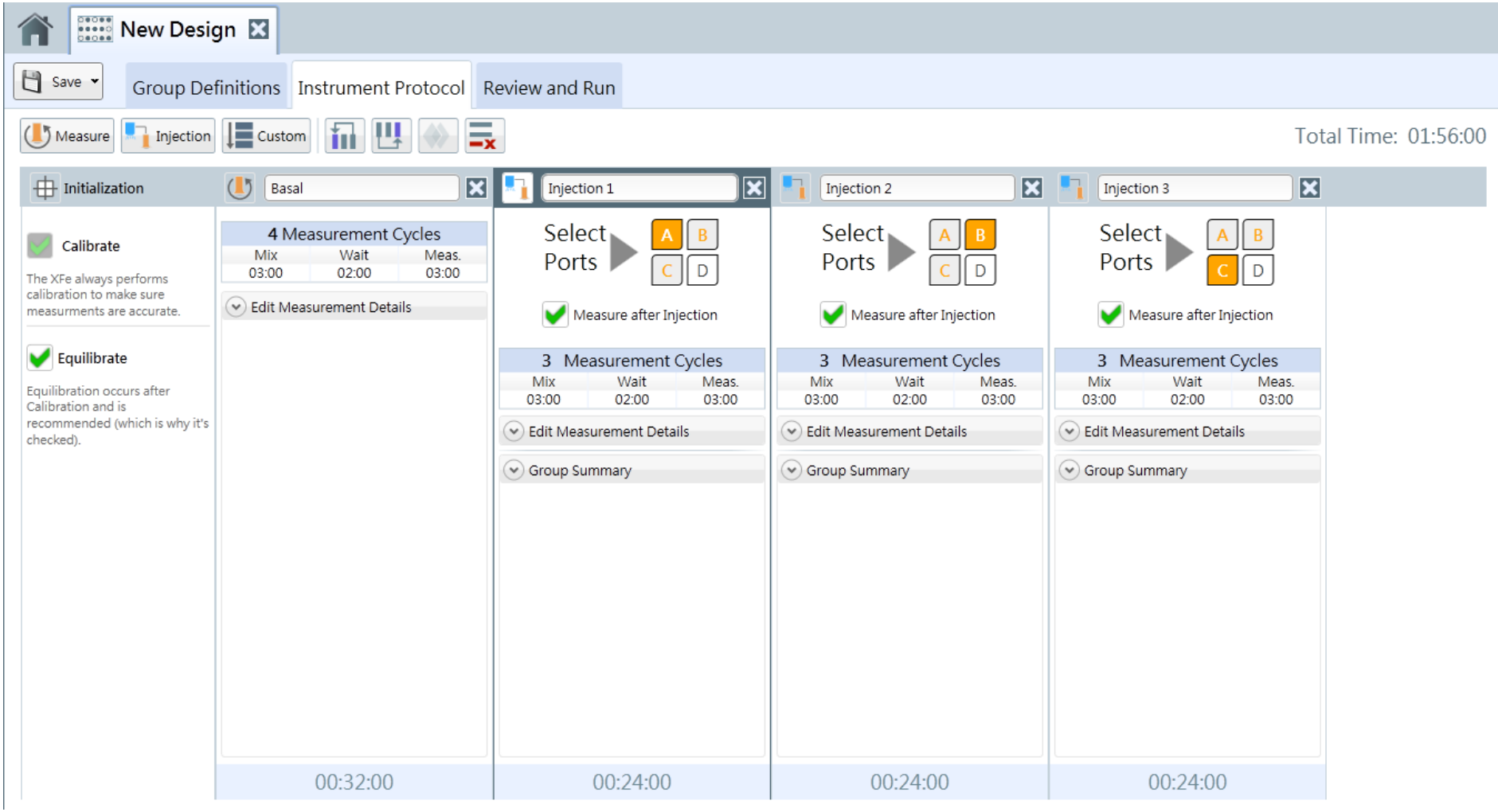
Assay Medium	Injection	Concentration in Port
500 uL	56uL	10X Compound A
556 uL	56 uL	11X Compound B
612 uL	56 uL	12X Compound C
668 uL	56 uL	13X Compound D
724 uL		





Design and Load Protocol

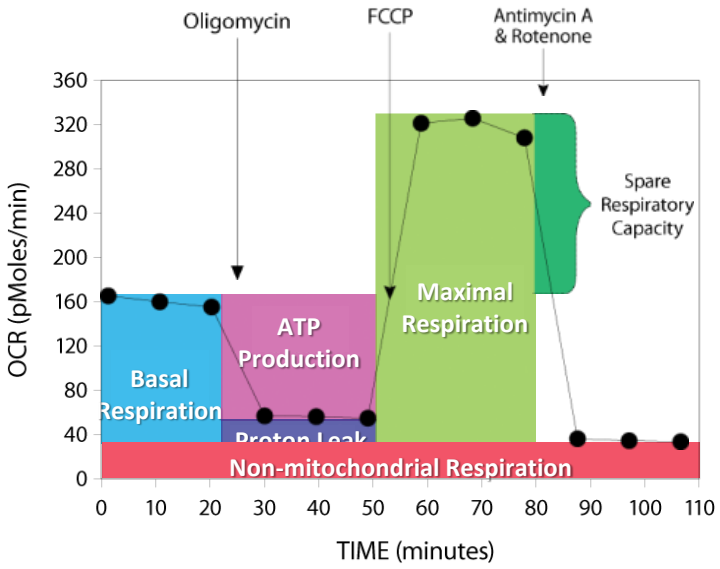
- **3 Steps complete assay design.**



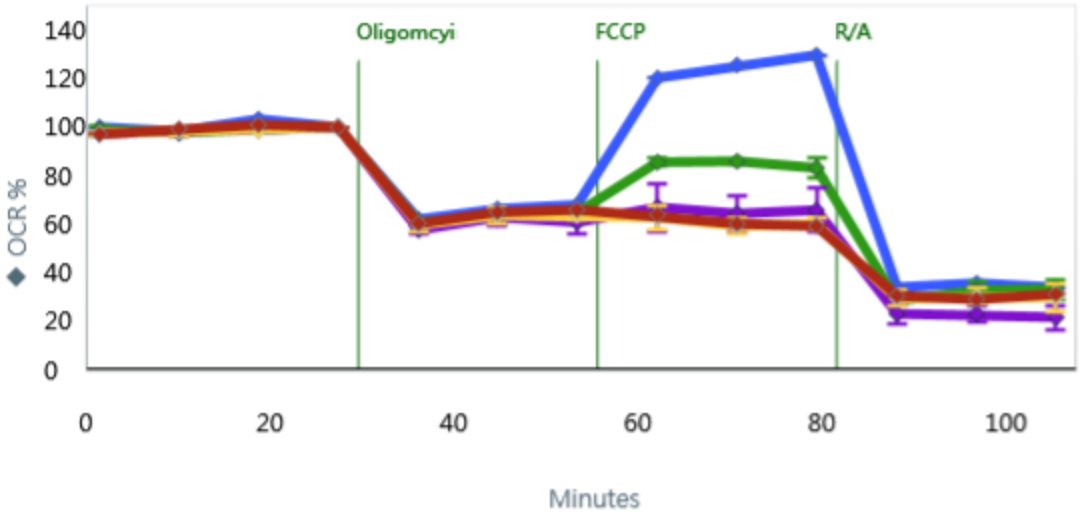
Run assay

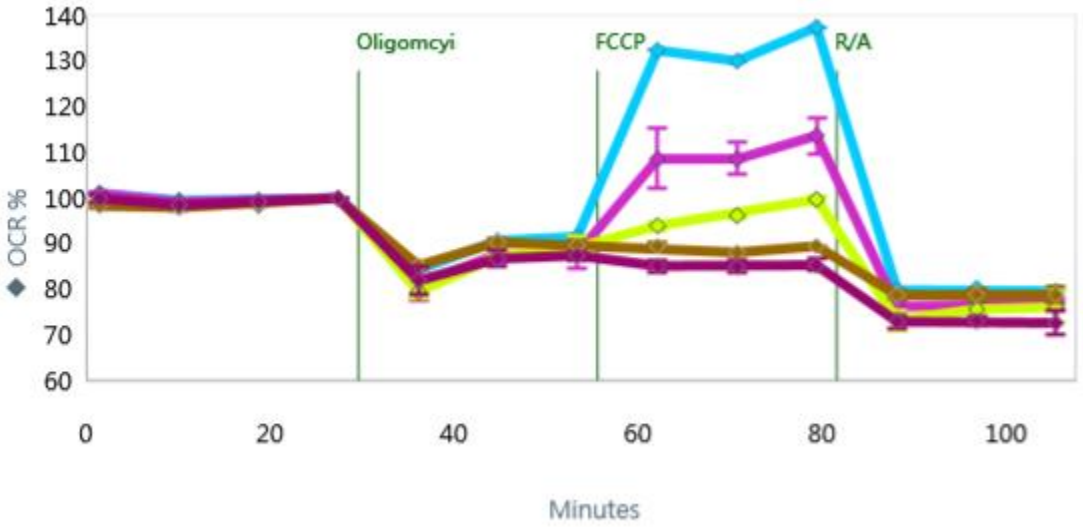
- **Put on the plate with right direction.**
- **Barcode toward the front**

FCCP Titeration Test



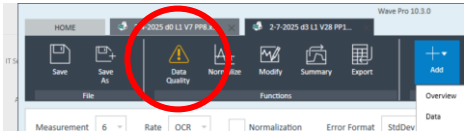
XF^{e} Mito Stress Test

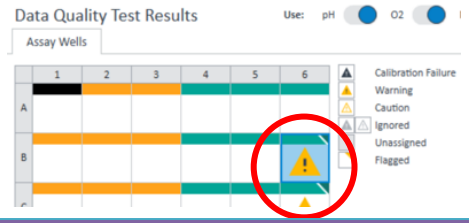


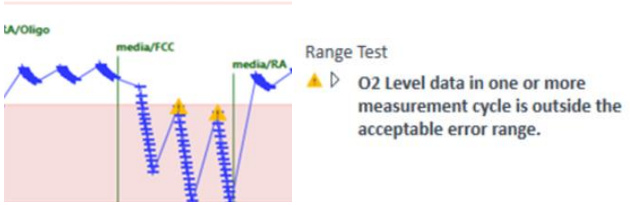


Seahorse Analytics and Wave Pro

- Automated data QC







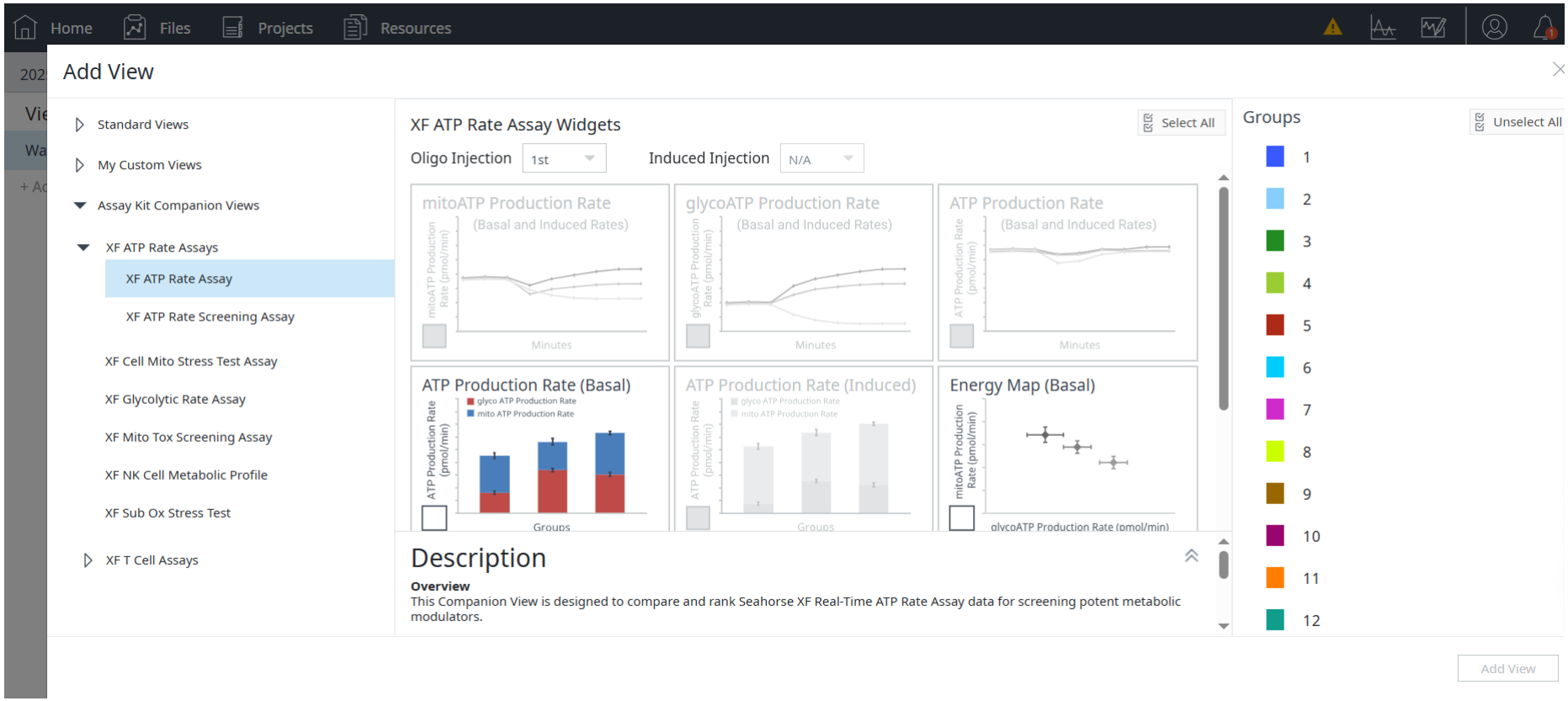
Test applied when selecting "Data Quality" icon:	XF V7	XF V28	XF Flex 3D Capture plate
Background test • Identifies unexpected data changes (sudden or drifting) – only O_2 • Identifies cells present in background wells	✓	✓	✓
Level tests • Data range checks • Identifies sudden data changes	✓	✓	✓
Rate test • Lower Limit of Detection check	✓	✓	–
Injection test (2 test sets) • Identifies injection failures • Identifies injection problems	✓	✓	–
Temperature test • Verifies if the instrument set point temperature matches the actual tray temperature during an assay	✓	✓	✓
Calibration test (performed on all wells) • Identifies which wells failed calibration: O2 or pH or both	✓	✓	✓

Agilent Seahorse Analytics
Report Generator

(Any PC)

Wave Pro
(Windows only)

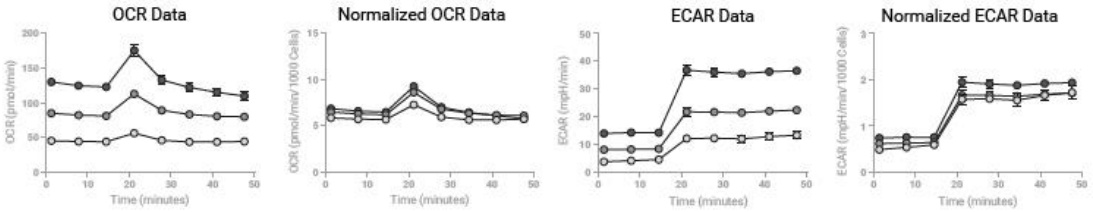
DATA ANALYSIS



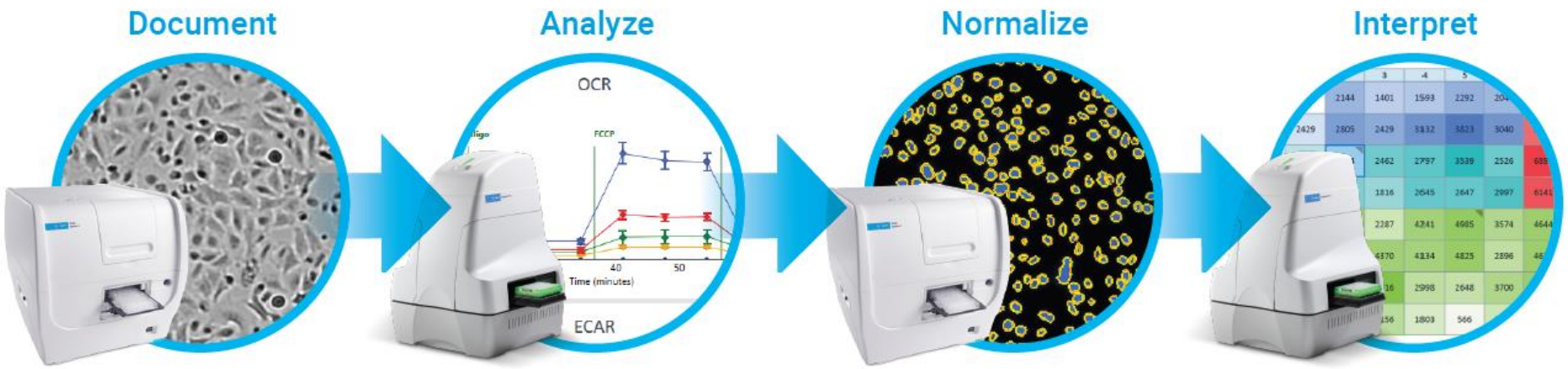
https://www.agilent.com/en/product/cell-analysis/real-time-cell-metabolic-analysis/xf-software/agilent-seahorse-analytics-787485

Normalization

- 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.



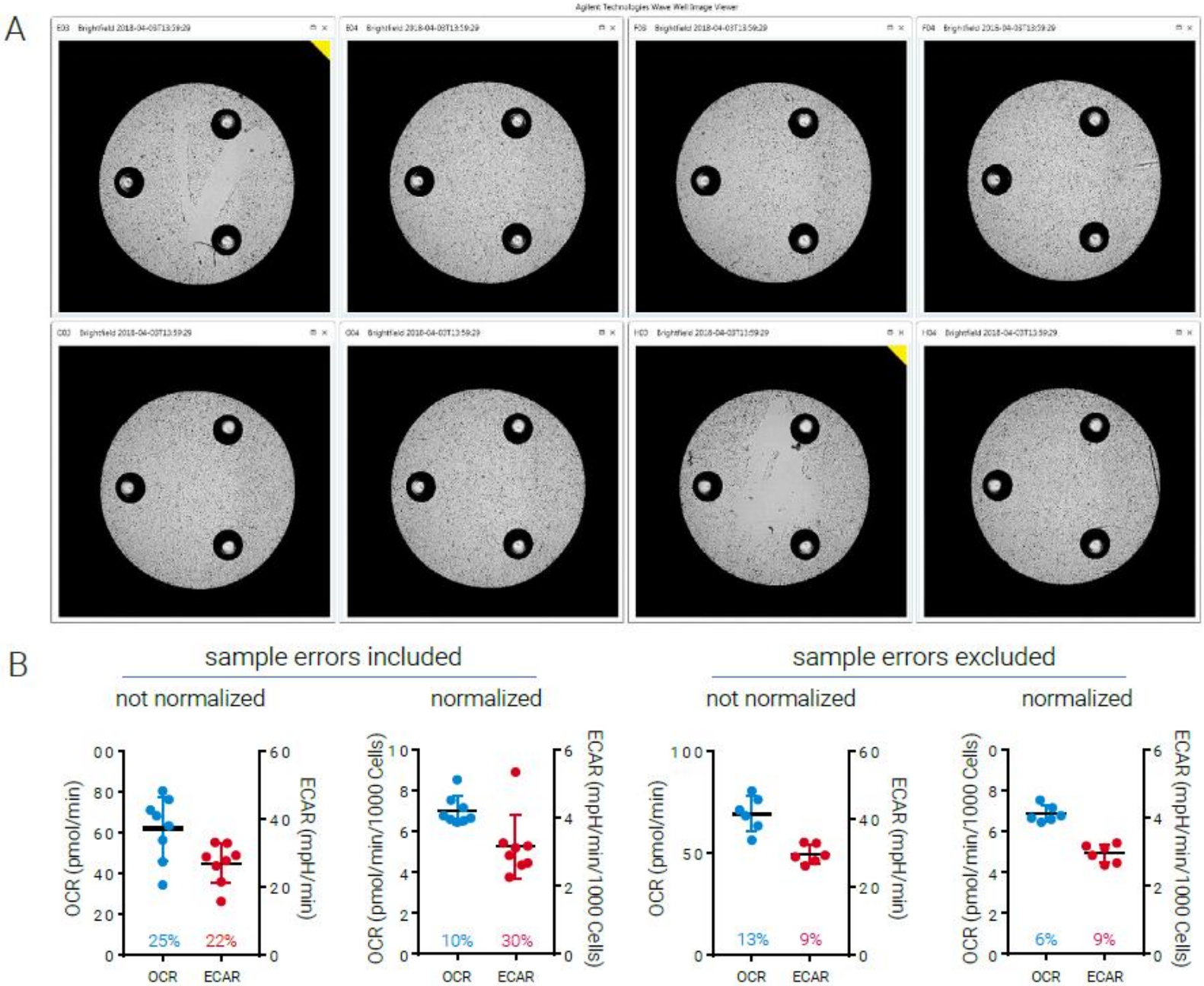


Figure 1. Error identification and documentation for XF analysis using brightfield images. A) Whole well brightfield images of A549 cells are compared by using the "well comparison" function in Wave software. Wells experiencing cell loss are marked by yellow flags on the upper right corner. B) Basal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

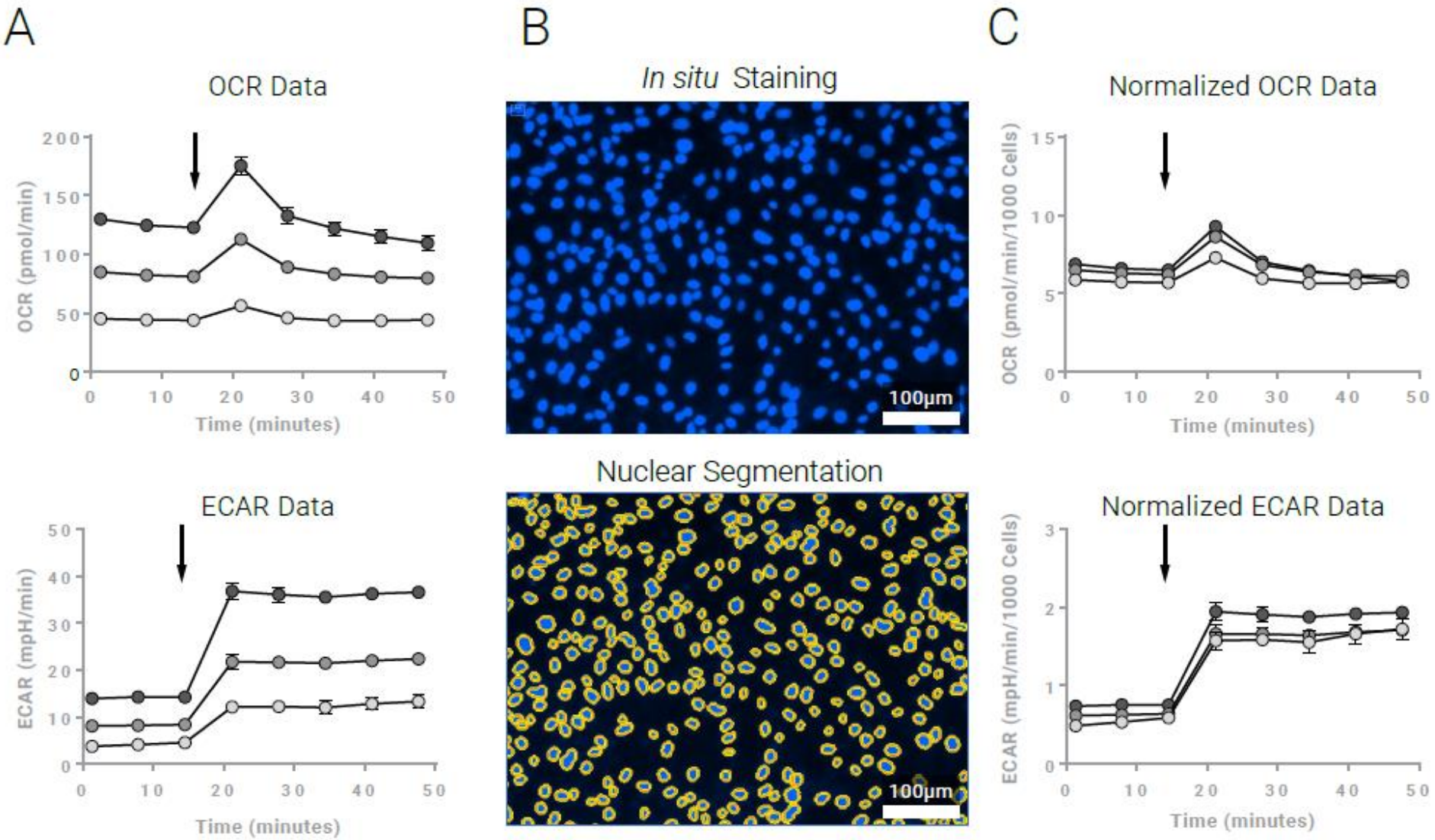


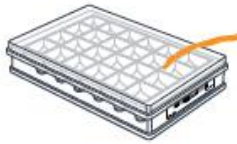
Figure 2. Example of XF data normalization using *in situ* nuclear staining and *in situ* cell counting. SKOV3 cells were plated at 1x10^4, 2x10^4, 3x10^4 cells per well, cultured 24 h, subject to the XF Cell Energy Phenotype Test and followed by image analysis. A) Raw OCR and ECAR change with injection (arrows) of oligomycin + FCCP (1.0 µM and 0.5 µM final, respectively), including 20 µM Hoechst 33342 (2 µM 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 ± SD, n=4).


Plate Name: 012318_pheno_kit
Image Sets
Brightfield 2018-01-23T09:13:12
Well Image: C05
Assay File Size:
21.82 MB (193 images)
Zoom
Sharpness
Brightness
Compare Sets
Remove Image
Import Image
Import Selected
Compare Wells
37.0° C
Agilent Technologies

Imaging and Cell Counting Workflow
1.18.18

3-4 days is typical to complete this workflow.

1 Seed Plate
Wait for cells to attach



2 Incubate Cells
Plate is incubated in a CO2 incubator until media exchange (30 minutes before running)

3 Media Exchange, Image & De-gas
Media in the cell plate is exchanged

4 Scan Barcode

5 Put plate in Cytation
Run Imaging and Cell Counting from Controller

6 Perform Brightfield Scan

Live preview of images during scanning. User can flag wells.

Image sat is stored on the Controller for access by Wave. User can alsc 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

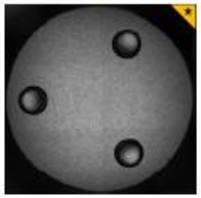
Calibration 20 minutes

- 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

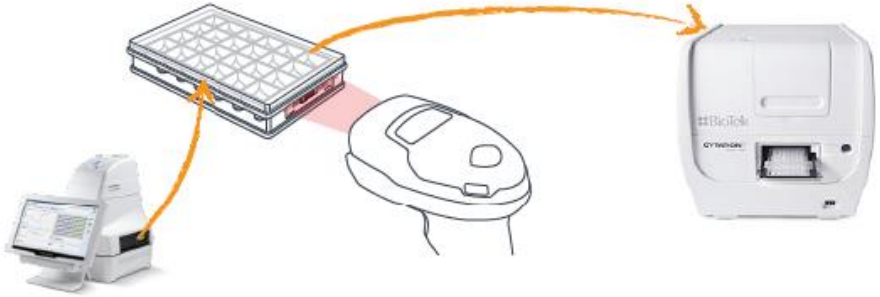


9 XF Run Complete

Remove stained plate from XF

10 Scan Barcode

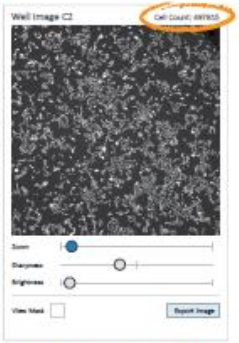
11 Put Plate in Cytation



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.

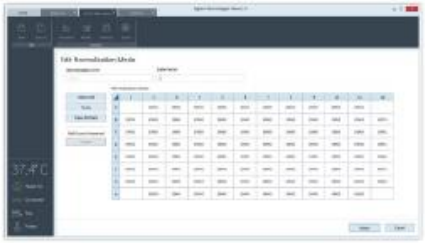
14 Scan Complete. Go to Wave

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

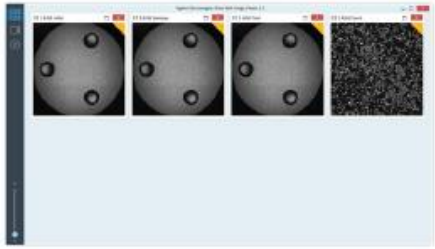
User can add Images View from the finished assay ribbon.

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

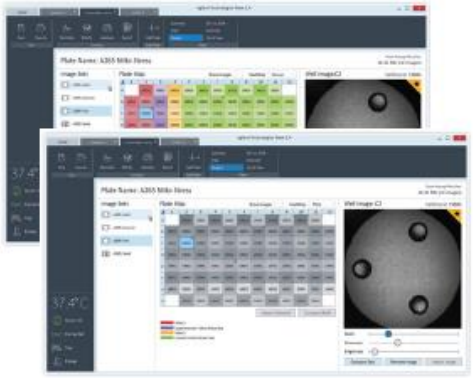
User can compare multiple images from within a set and get up close on specific images and their data.

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 Embeding

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 embeded into the ASYR file. The Assay can be taken to Wave Desktop for analysis with embedded images and cell counts.

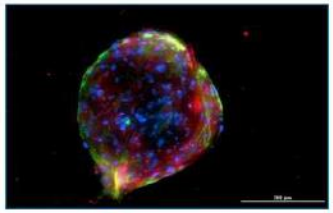
19 Manage

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

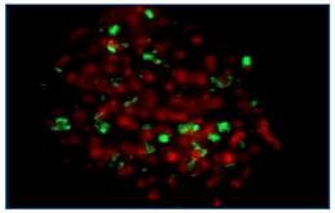
User can:

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

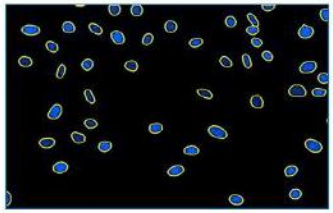
Cytation更多實驗應用



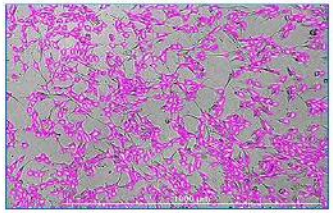
3D細胞培/類器官



細胞凋亡

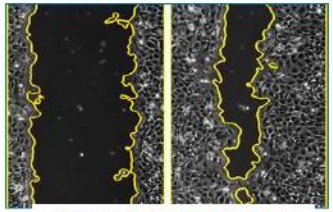


細胞計數

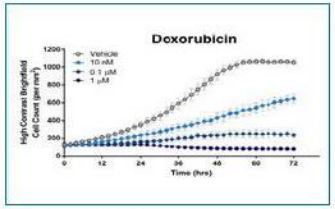


無標記細胞計數

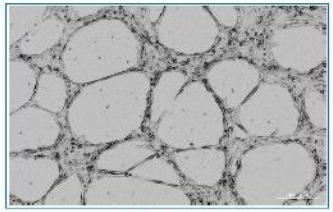
Elispot



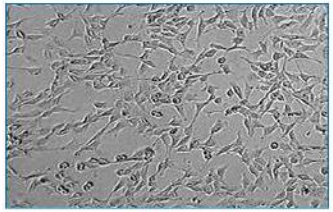
細胞劃痕/細胞遷移



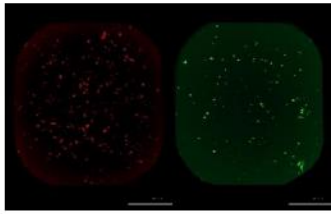
細胞增殖/活力/毒性



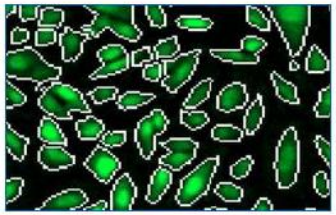
血管生成



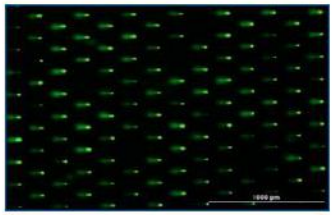
細胞覆蓋率



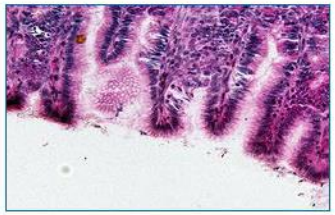
中和抗體檢測



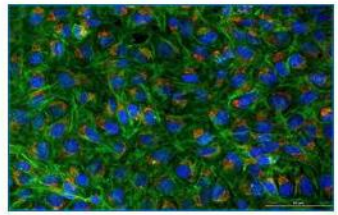
鈣離子檢測/快速動力學影像



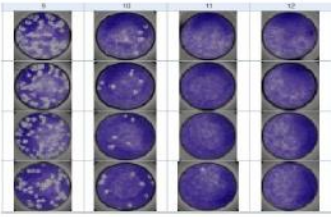
基因毒性/DNA損傷



組織學



細胞/組織免疫螢光



空斑檢測
Plaque Assay