



Keys to Success for XF Assays



Presentation Outline

XF Assay Flow Chart

Cell Culture and Seeding

Cell Seeding Density

XF Cell Stress Test Compound Titration



XF Assay Flow Chart

Prepare cells in XF plate



Seed cells and incubate overnight in growth medium



Change medium to bicarbonate-free low-buffered assay medium



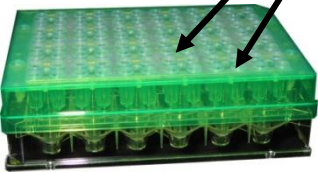
Prepare sensor cartridge



Hydrate sensor cartridge overnight



Add compounds to reagent ports



Assay



Calibrate sensors



Good Cell Culture Practice



Watch for morphology or growth changes

- Age
- Contamination

Passage before confluence

Ensure consistent media components

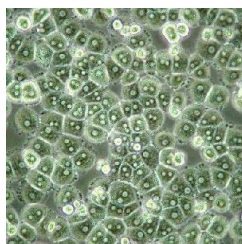
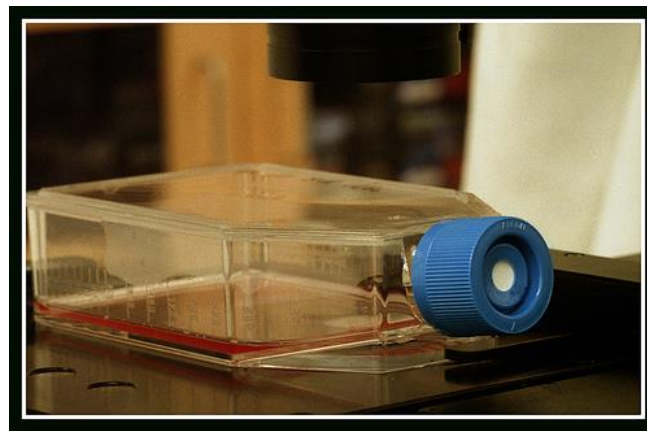
- Lot test serum
- Fresh reagents & kept dark

Monitor incubators

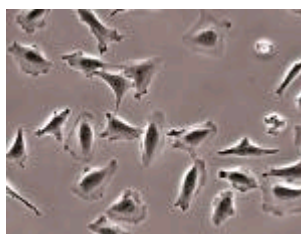
- Humidity
- CO₂

Factors dependent on your cell type

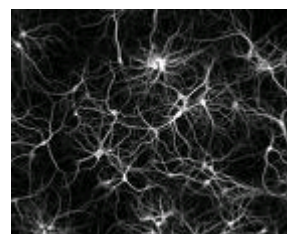
- Cell number
- Time in culture
- Cell line or primary cell
- Proliferating or differentiated
- Surface treatment
- Biological/physiological requirements



adipocytes



fibroblast



neurons

- Cell Reference Database
<https://www.agilent.com/cell-reference-database/>



XF Assay Flow Chart

Prepare cells in XF plate

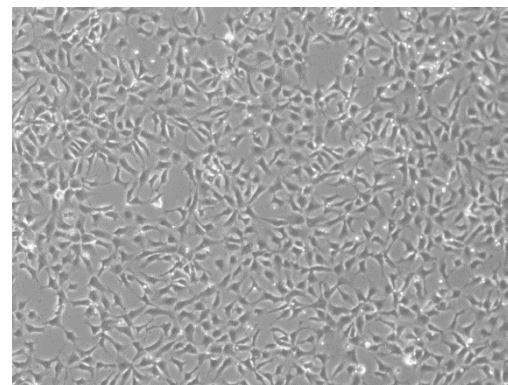


**Seed cells and
incubate overnight
in growth medium**



Key Factors in Cell Seeding

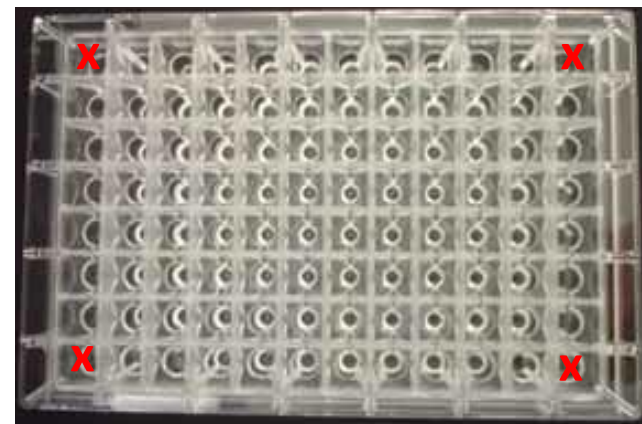
- Consistency
- Single cell suspension is optimal
- Consider cell attachment
- Minimize edge effect



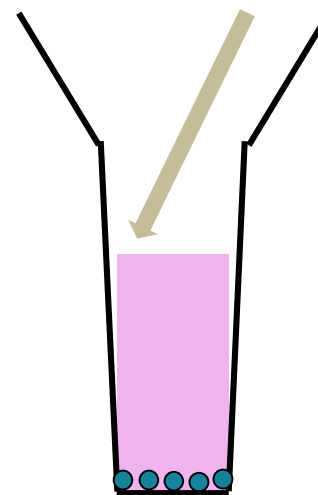
Neural blastoma cells

Cell Seeding on XF96 Microplate

- Seed 80 μL of cell suspension per well
- Allow plate to rest at room temperature in the tissue culture hood for one hour
- Allow cells to grow overnight in a cell culture incubator



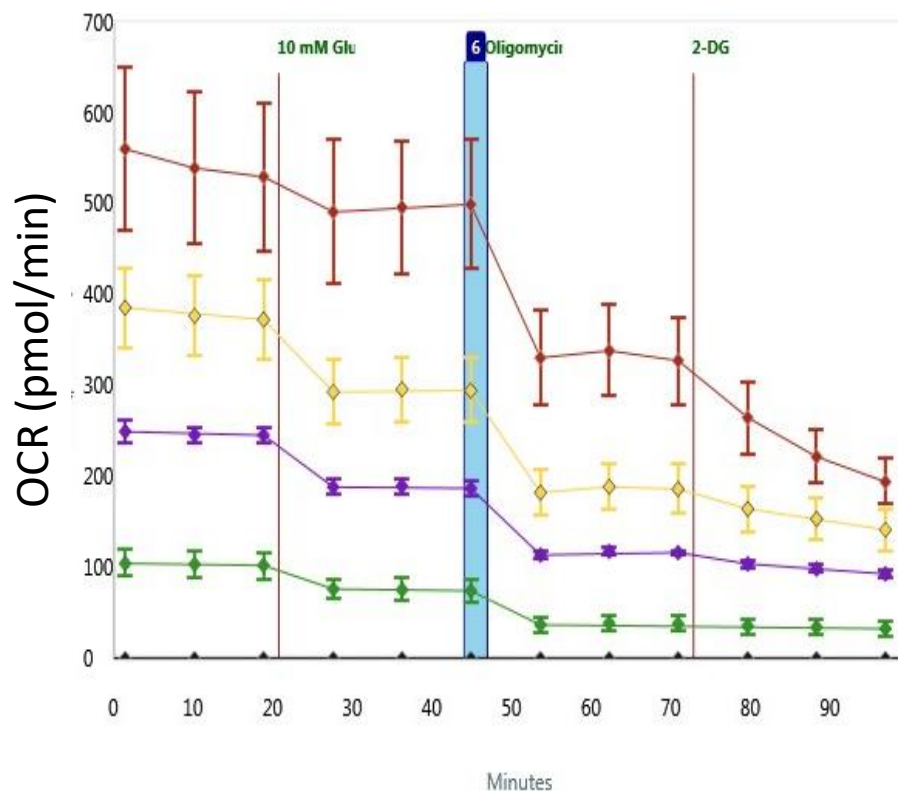
80 μL of cells



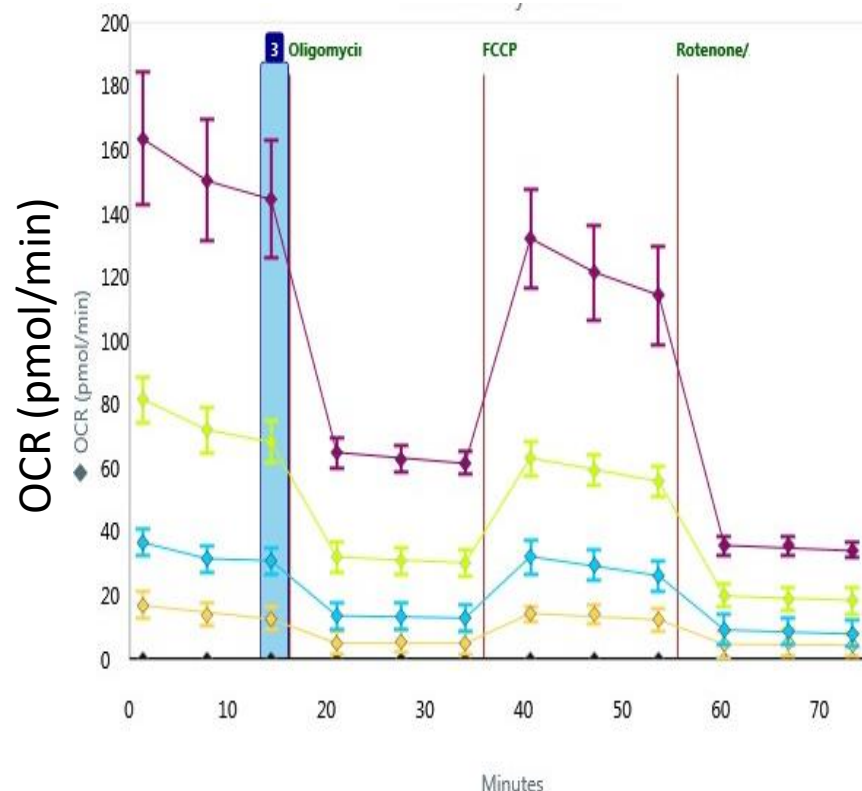
Criteria for Determining Optimal Cell Density

- Use OCR basal rates to determine optimal cell density
 - ❖ For Glycolysis Stress Test, use measurement before Oligomycin injection (6)
 - ❖ For Cell Mito Stress Test, use measurement before Oligomycin injection (3)

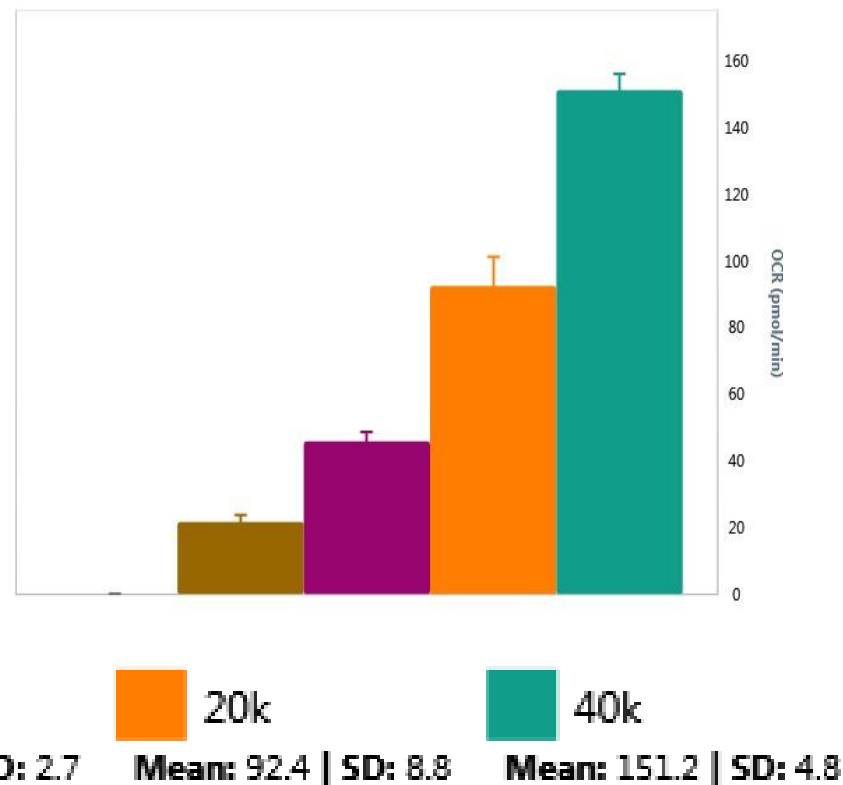
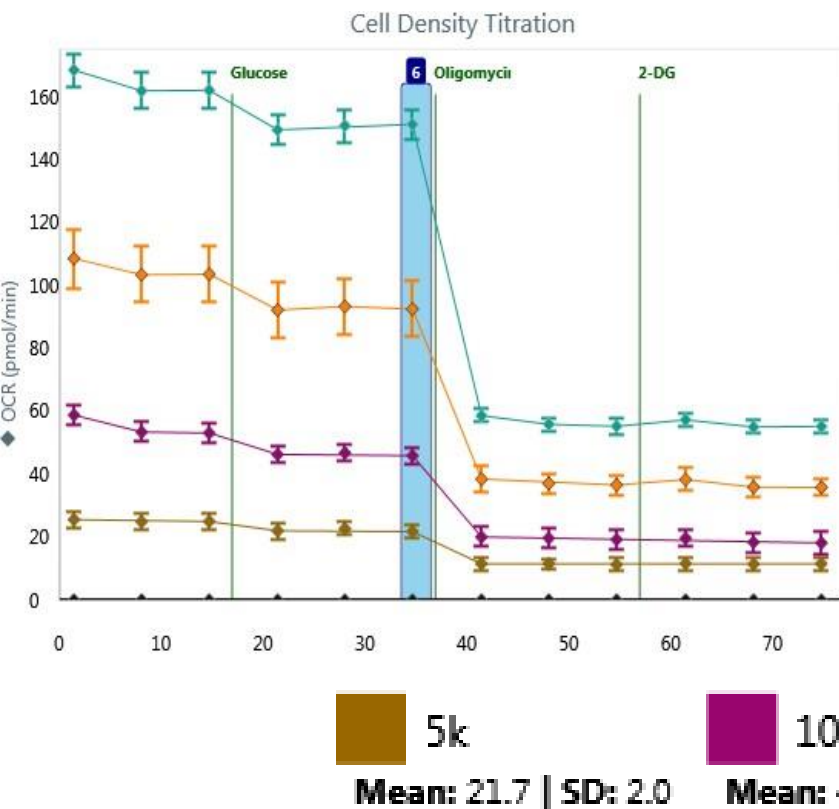
Glycolysis Stress Test



Cell Mito Stress Test



Determining Optimal Cell Density (XFe96)



- ❖ Good signal range– XFe96/XFe96: ~ 20 – 160 pmol/min
- ❖ Nice, consistent monolayer – not necessarily confluent
- ❖ Small magnitude of error
- ❖ Linear range of the cell type

XF Assay Flow Chart

Prepare cells in XF plate



Seed cells and
incubate overnight
in growth medium



Change medium to
bicarbonate-free
low-buffered assay medium



Day of Assay: Prepare Assay Medium

Start from XF Base Medium, add substrates fresh

Glycolysis Stress Test Assay Medium

NO sodium bicarbonate

Low phenol red (3 mg/L)

1. Add fresh Glutamine
(Not Glutamax)
2. Warm to 37°C
3. Adjust pH to 7.4



Cell Mito Stress Test Assay Medium

NO sodium bicarbonate

Low phenol red (3 mg/L)

1. Add substrates such as:
 - Glucose
 - Pyruvate
 - Glutamine
2. Warm to 37°C
3. Adjust pH to 7.4



Day of Assay: Prepare Assay Medium

pH-ready XF media and supplements

Catalog number	Description	Compatible with
103575-100	Seahorse XF DMEM Medium, pH 7.4, 500 mL	All XF assay kits. All Analyzers except for XF24 Analyzer ¹ .
103576-100	Seahorse XF RPMI Medium, pH 7.4, 500 mL	All XF assay kits. All Analyzer except for XF24 Analyzer ¹ .
103577-100	Seahorse XF 1.0 M Glucose Solution, 50 mL	All XF assay kits. All Analyzers.
103578-100	Seahorse XF 100 mM Pyruvate Solution, 50 mL	All XF assay kits. All Analyzers.
103579-100	Seahorse XF 200 mM Glutamine Solution, 50 mL	All XF assay kits. All Analyzers.



XF Assay Flow Chart – Adherent Cells

Day Before Assay*



Seed cells and incubate overnight* in growth medium



Change medium to prepared assay medium



1 hr
37°C
No CO₂

* Or longer, depending on cell type



XF Assay Flow Chart – Suspension Cells

Same Day as Assay



Wash cells into
prepared assay
medium



Count and Seed
cells in XF Cell
Culture Microplate

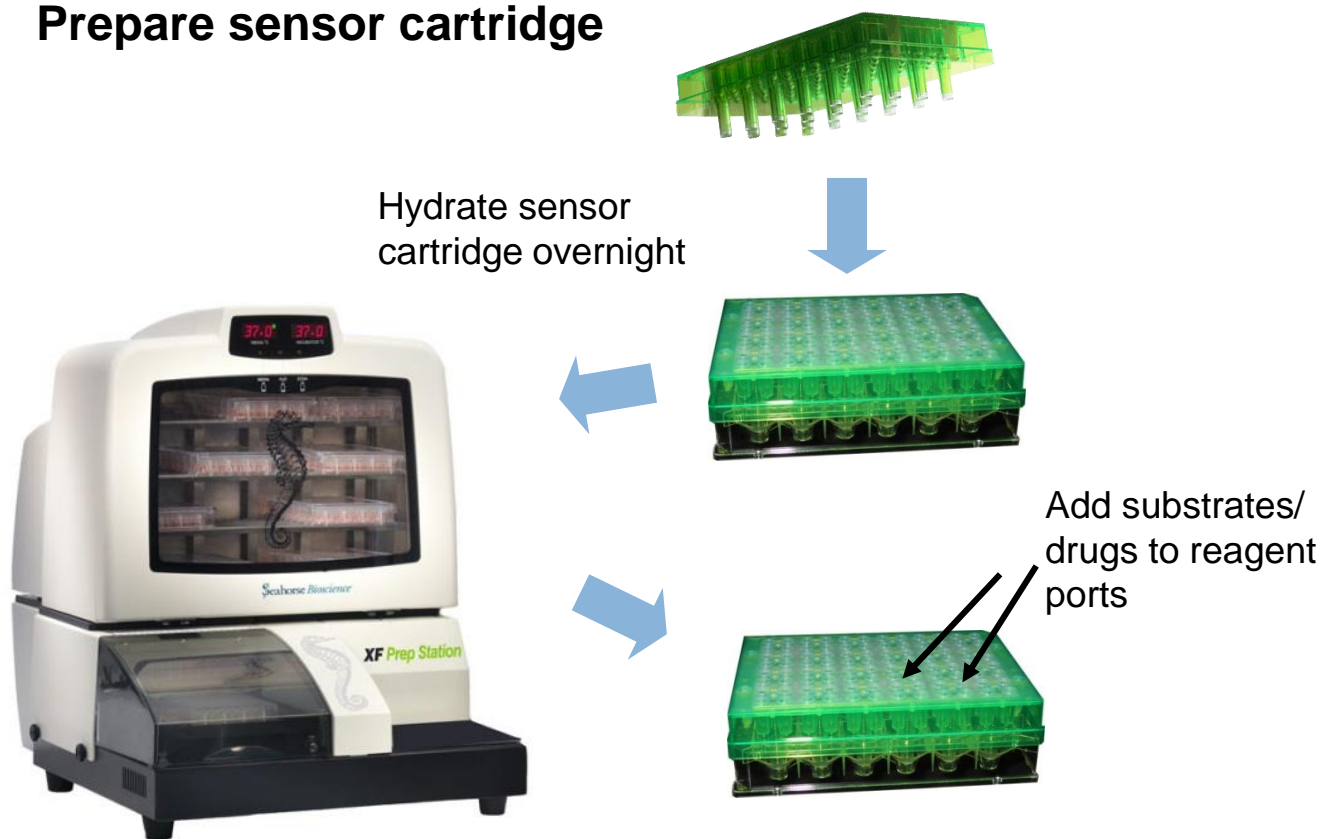


1 hr
37°C
No CO₂

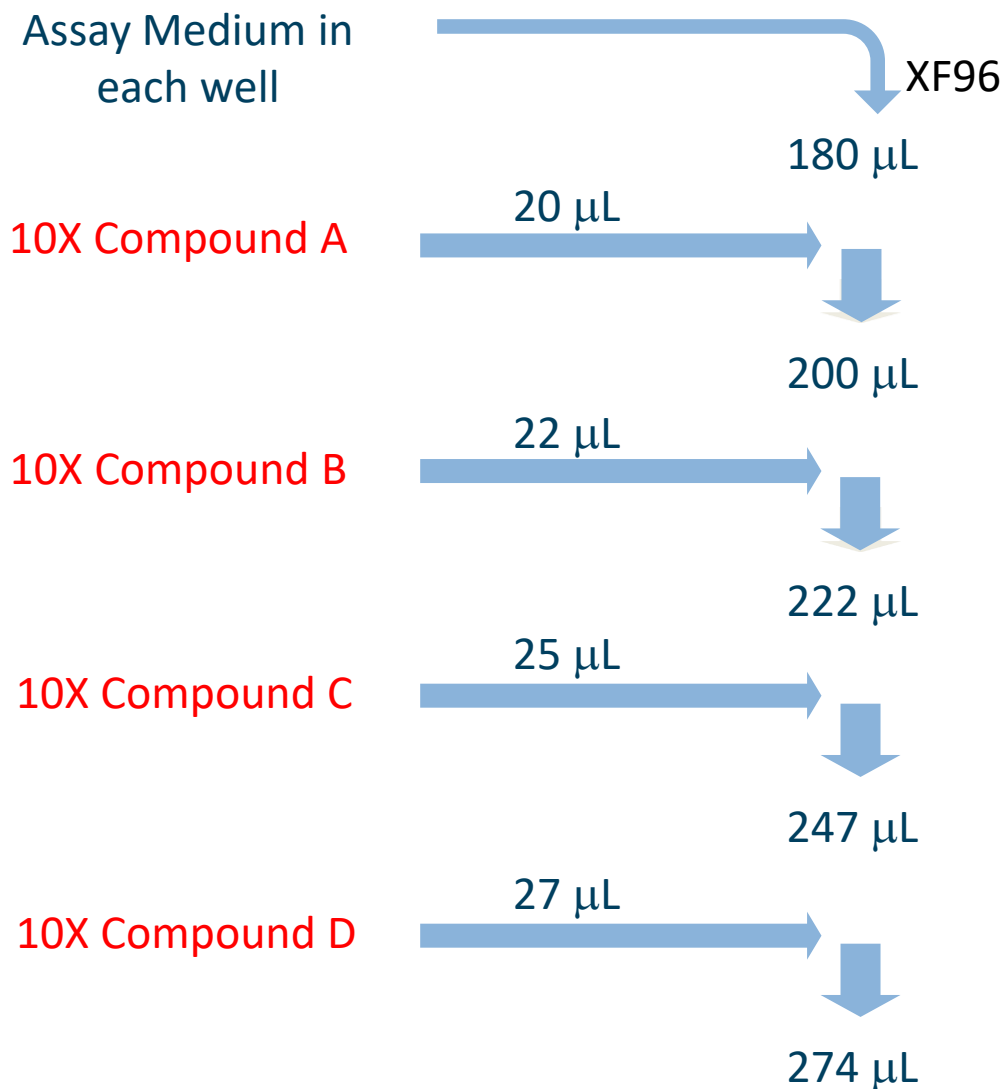


XF Assay Flow Chart- Cartridge

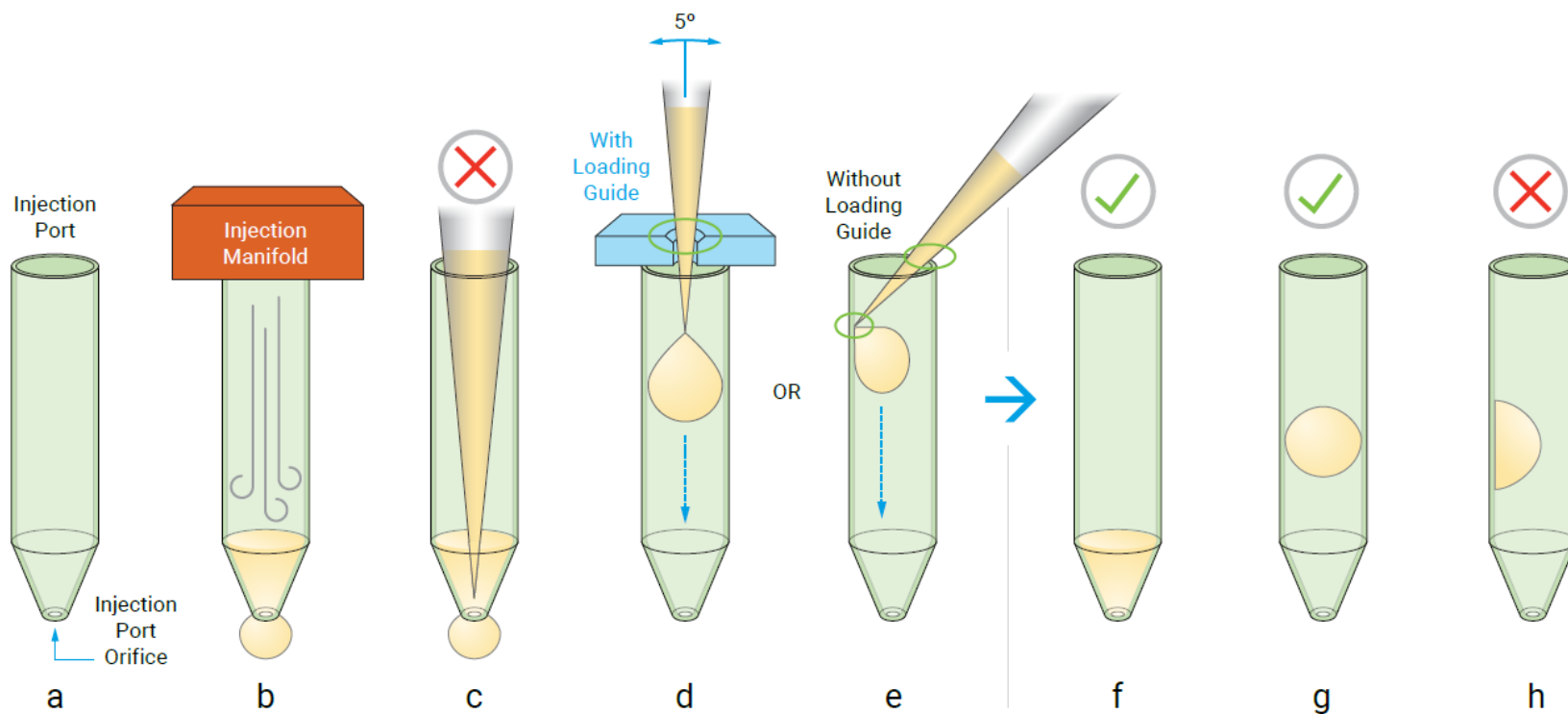
Prepare sensor cartridge



Making Stock Compounds: Constant Stock

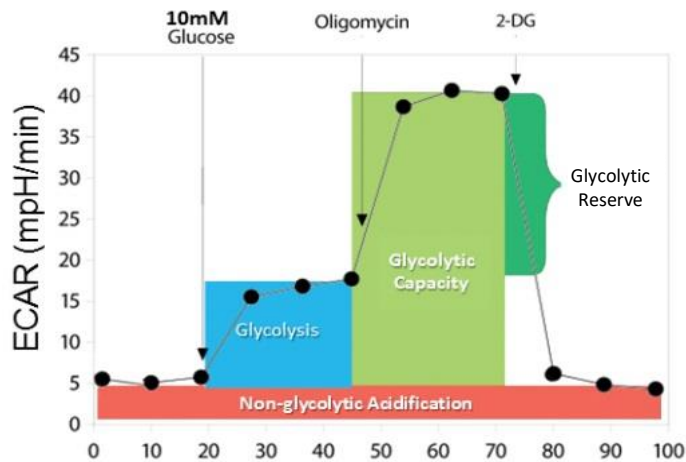


Injection Port Mechanism and Proper Port Loading Techniques

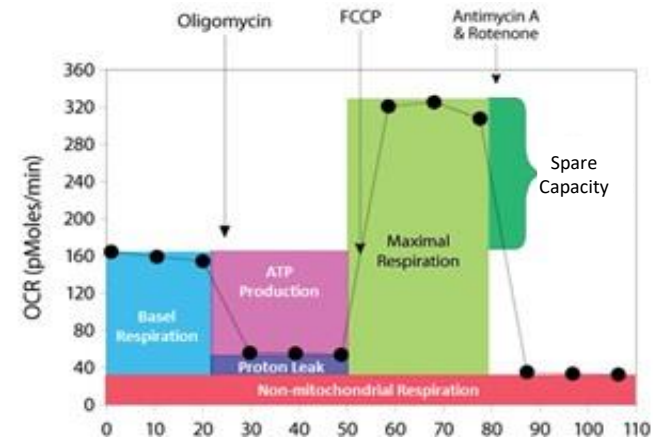


Characterization of New Cell Types for XF Stress Test Kits

- Glycolysis Stress Test
 - Cell Seeding Density



- Cell Mito Stress Test
 - Cell Seeding Density
 - FCCP Concentration



Characterization of New Cell Types

What we've learned...

- From:
 - 5000+ publications
 - Eight years internal development and experience
- About:
 - Optimal cell density
 - Typical oligomycin concentration
 - Range of FCCP efficacy
 - Acceptable ranges for OCR and ECAR

allows us to simplify the workflow

- Cell Density:
 - Literature provides assay range
 - Visual assessment is usually sufficient
- Oligomycin:
 - 1.5 μM works for most cell types
- FCCP:
 - Optimal concentration is almost always in the range of 0.2-2.0 μM *

* higher concentrations are used when BSA or serum is present



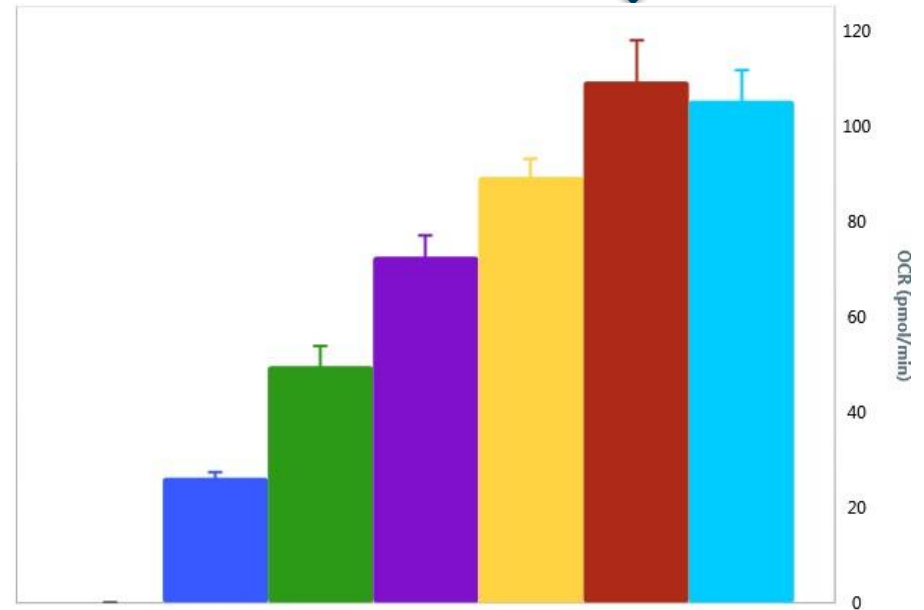
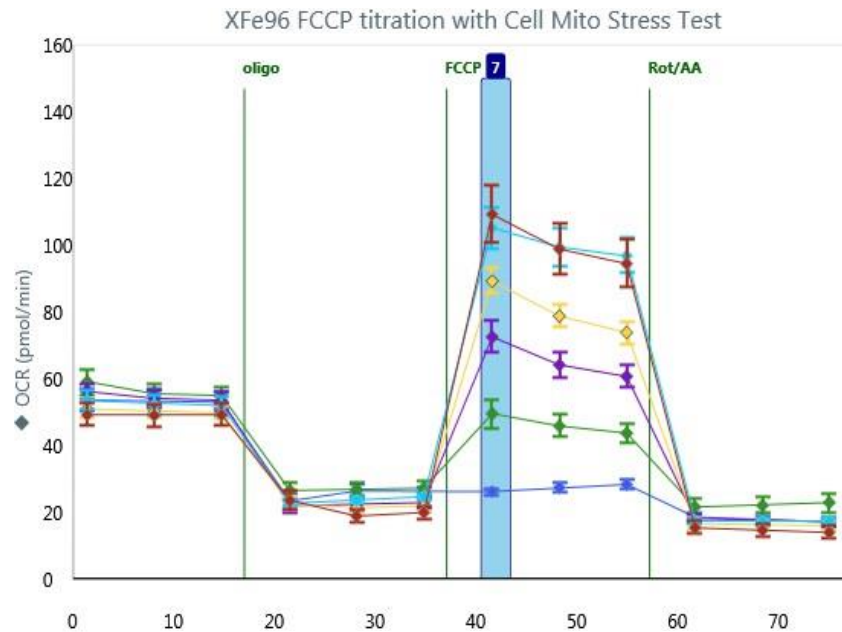
Cell Density

	A	B	C	D	E	F	G	H	I	J
1	Title	Authors	Journal	Publica	Cell Line	Cell Type	Speci	XF Form	XF Assay	Seeding density
4	Activation of pattern recognition receptors in brown adipocytes induces inflammation and suppresses uncoupling protein 1 expression and mitochondrial respiration	Bae J, Ricciardi CJ, Esposito D, Komarnytsky S, Hu P, Curry BJ, Brown PL, Gao Z, Biggerstaff JP, Chen J, Zhao L.	Am J Physiol Cell Physiol	May-14	brown adipocytes	Adipocytes	Mouse	24	Substrate utilization	2.5x10 ⁴ cells/well
10	GADD45 ³ regulates the thermogenic capacity of brown adipose tissue	Gantner ML, Hazen BC, Konkright J, Kralli A	Proc Natl Acad Sci U S A	Aug-14	adipocytes	Adipocytes	Mouse	96	Substrate utilization	4.0x10 ³ cells/well
11	Irisin and FGF10 regulate brown adipocyte function in HFD mice							24-3	Substrate utilization	5.0x10 ⁴ cells/well
12	Measuring respiration in brown adipocytes in real time							24	Cell Mitochondrial Stress Test	1.3x10 ⁴ cells/well
13	Mitochondria Regulates Adipocyte Differentiation in Mesenchymal Stem Cells							24	Cell Mitochondrial Stress Test	1.0x10 ⁵ cells/well
19	Thiazolidinediones are acute, specific inhibitors of the mitochondrial pyruvate carrier	Divakaruni AS, Wiley SE, Rogers GW, Andreyev AY, Petrosyan S, Loviscach M, Wall EA, Yadava N, Heuck AP, Ferrick DA, Henry RR, McDonald WG, Colca JR, Simon MI, Ciaraldi TP, Murphy AN	PNAS	Apr-13	brown	Adipocytes	Rat	24	Basal Metabolic Assay	2.0x10 ⁵ cells/well
20	Thiazolidinediones are acute, specific inhibitors of the mitochondrial pyruvate carrier	Divakaruni AS, Wiley SE, Rogers GW, Andreyev AY, Petrosyan S, Loviscach M, Wall EA, Yadava N, Heuck AP, Ferrick DA, Henry RR, McDonald WG, Colca JR, Simon MI, Ciaraldi TP, Murphy AN	PNAS					96	Basal Metabolic Assay	8.0x10 ⁴ cells/well

- Use customer knowledge & Seahorse Cell Line Database to identify optimal density range
- <https://www.agilent.com/cell-reference-database/>
- Seed 3 plates: cover range or best guess +/- 50%
- Visual inspection of cell density

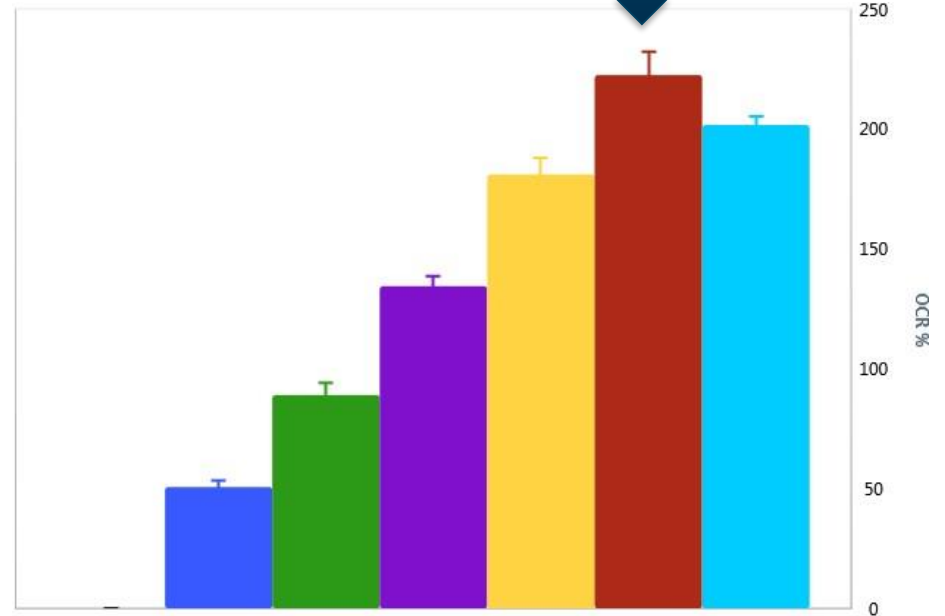
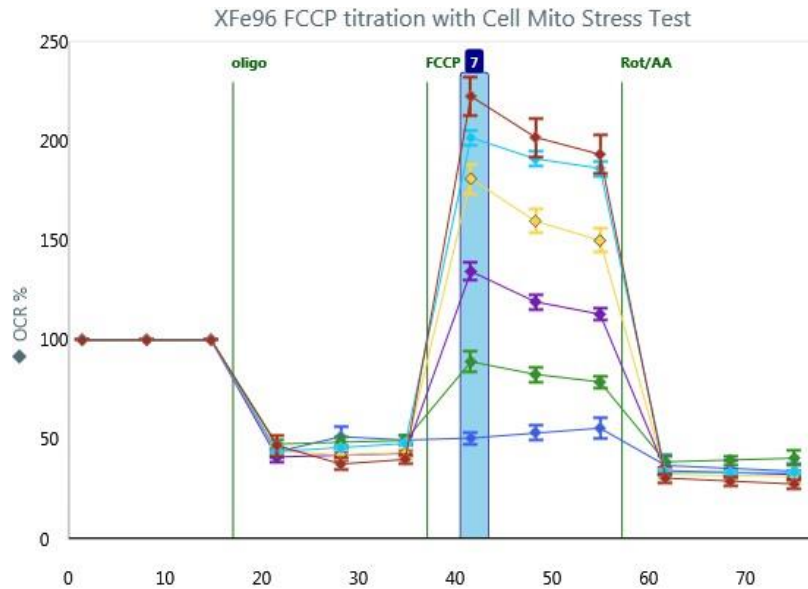


Determining Optimal FCCP Concentration



- ❖ Maximal stimulation of OCR is observed
- ❖ The lowest concentration of FCCP that produces maximal stimulation of OCR
- ❖ Ideally the OCR is stable over 3 measurement periods

Determining Optimal FCCP Concentration



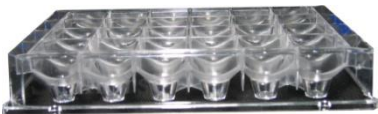
- ❖ Maximal stimulation of OCR is observed
- ❖ The lowest concentration of FCCP that produces maximal stimulation of OCR
- ❖ Ideally the OCR is stable over 3 measurement periods

XF Assay Flow Chart

Prepare cells in XF plate



Seed or Wash cells
in assay medium



Assay

Real-time data acquisition
and output

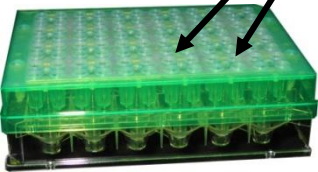
Prepare sensor cartridge



Hydrate sensor
cartridge overnight



Add compounds
to reagent ports



Calibrate sensors

