Technical meeting

Introduction of Seahorse® technology for studying the metabolism

Agilent Technologies

SeaHorse® device

Seahorse device is equipped with fluorescent probes allowing the measurement of:

- Oxygen consumption rate (OCR) 532/650 nm
- Extracellular acidification rate (ECAR) 470/530 nm

A large variety of kits:

- Mito Stress Test: mitochondrial respiration
- Glycolysis Stress Test: glycolysis

- XF Real-Time ATP Rate
- XF palmitate-BSA FAO Subbstrate
- XF Cell Energy Phenotype

Possibility to modulate temperature and measurement times

Agilent Seahorse XFp Analyzer

Agilent Seahorse XFe96 Analyzer

Agilent Seahorse XFe24 Analyzer

SeaHorse® device

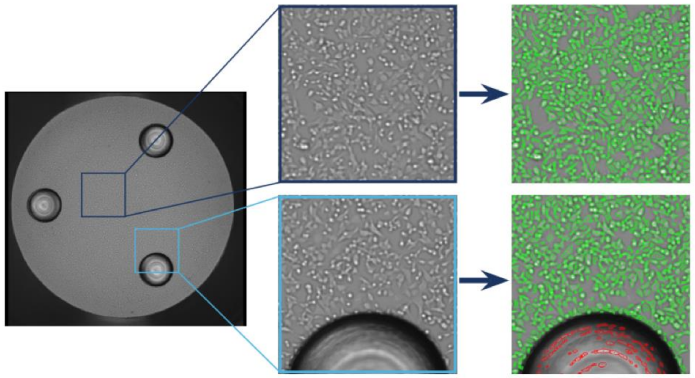
In our laboratory, we have the Seahorse XFe24 analyzer

Sensitivity from 10,000 cells per well

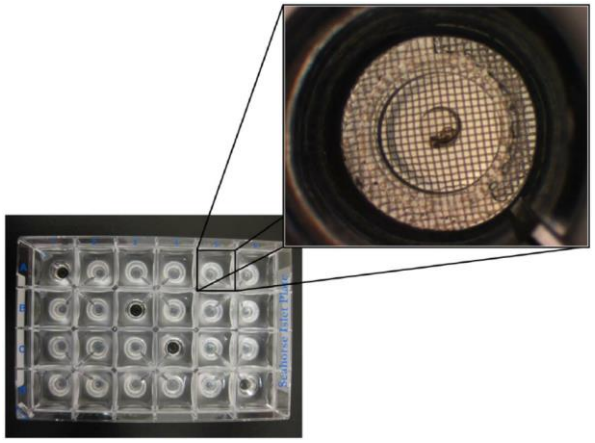
This measurement can be done on different types of sample:

- Cell lines
- Tissues
- Sphéroide
- Zebrafish

} Islet plate

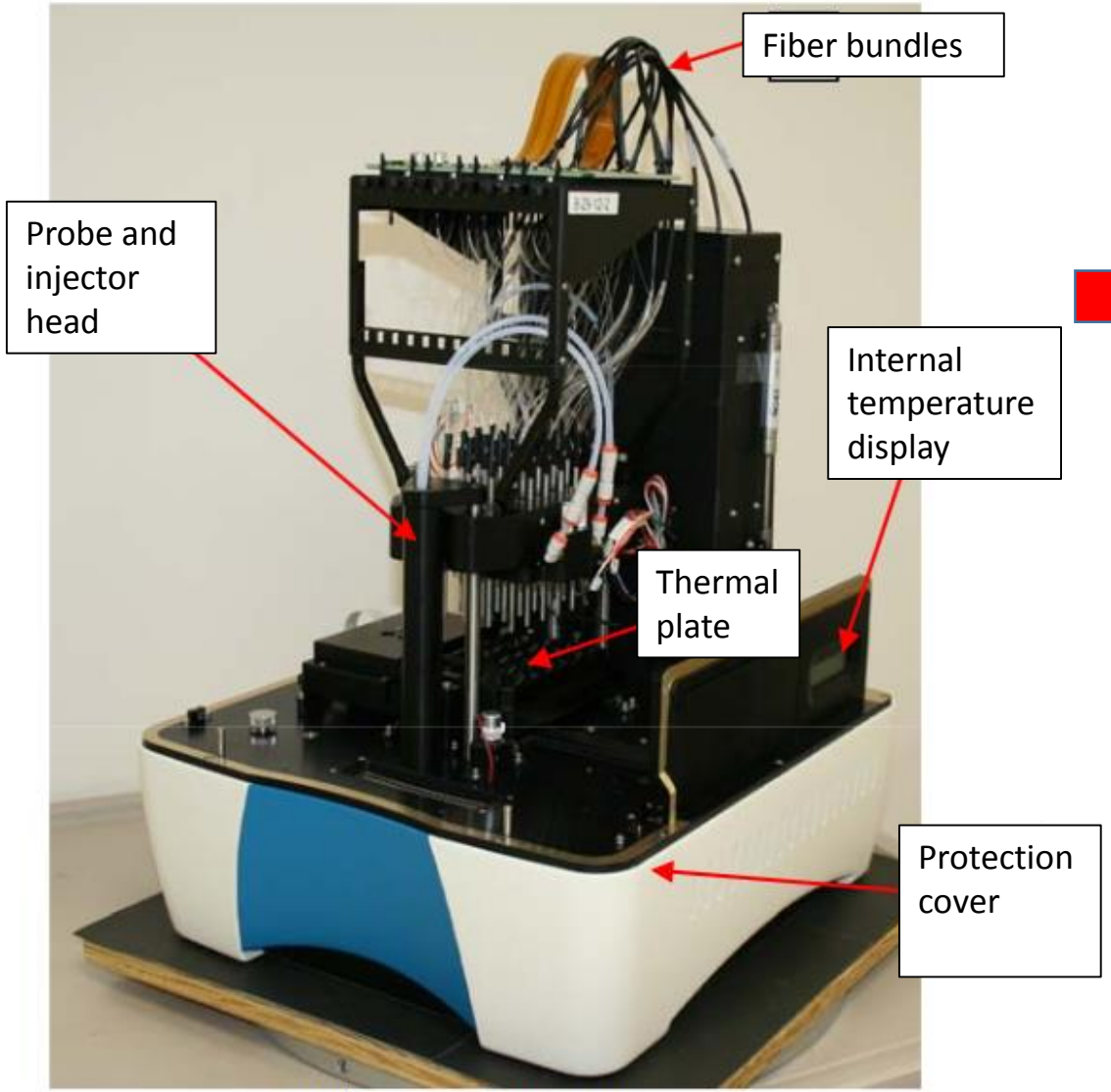


Standard plate

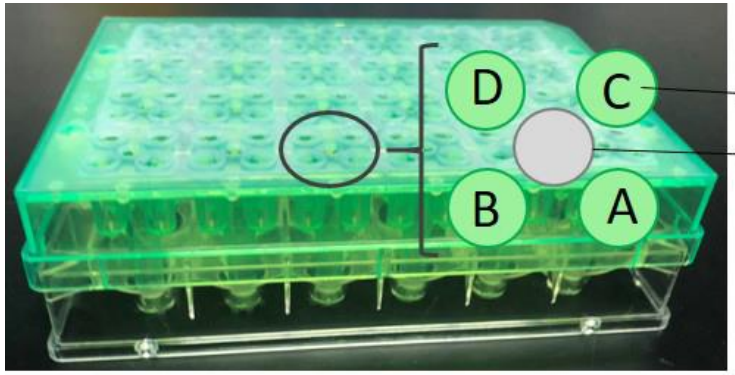


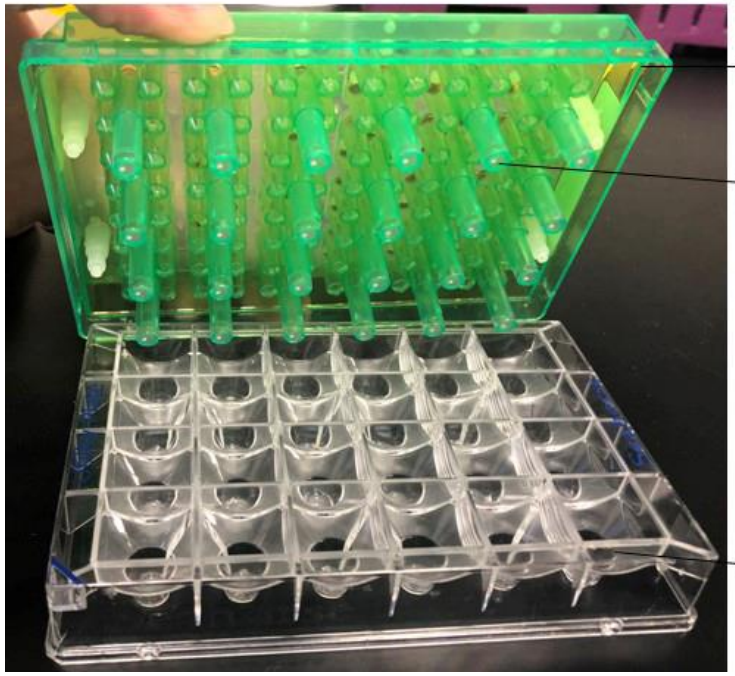
Islet plate

SeaHorse® device

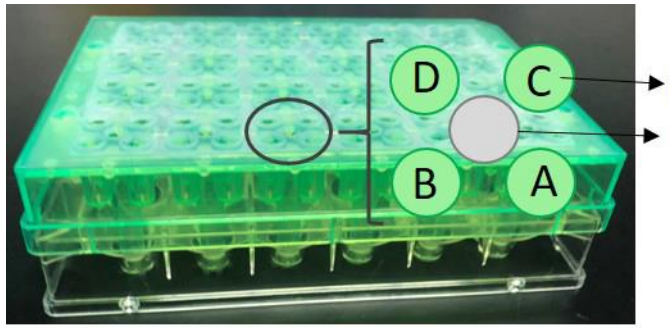


XF^{e} Front/Side View



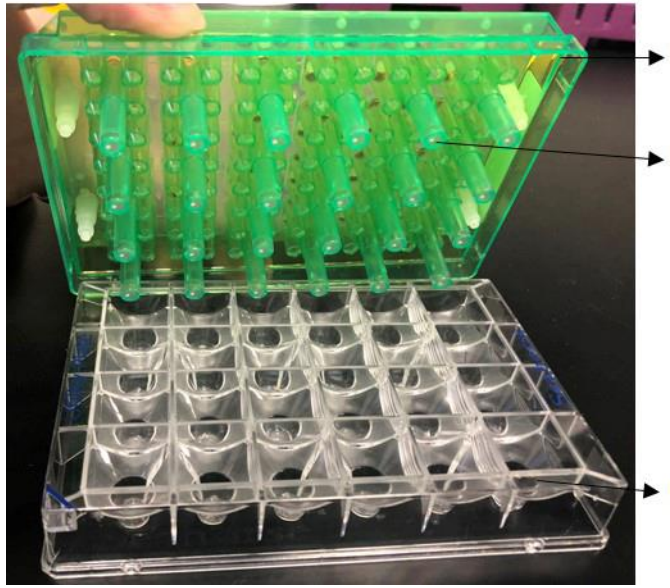


SeaHorse® device



injection port

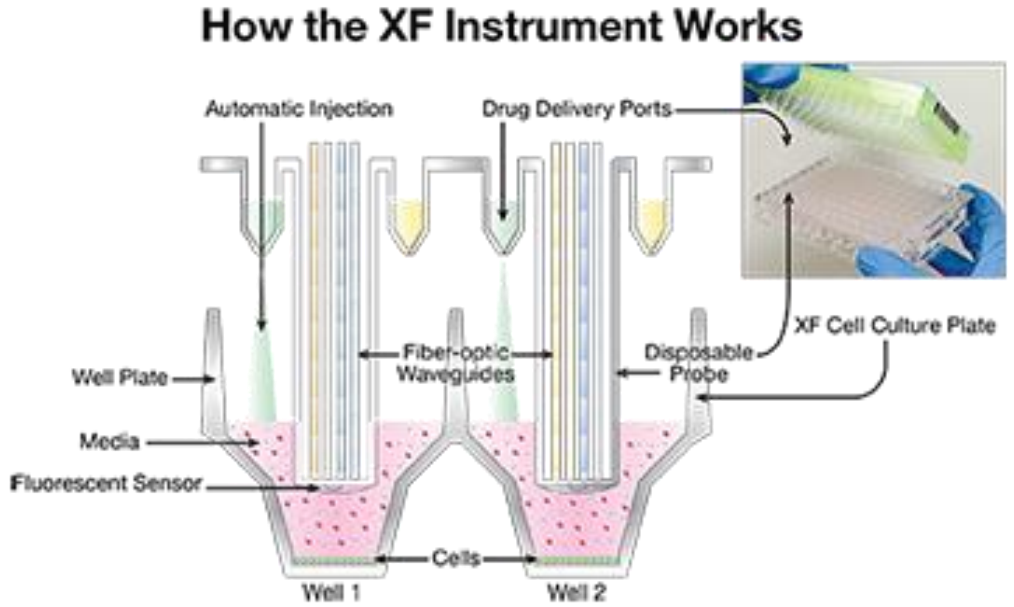
sensor

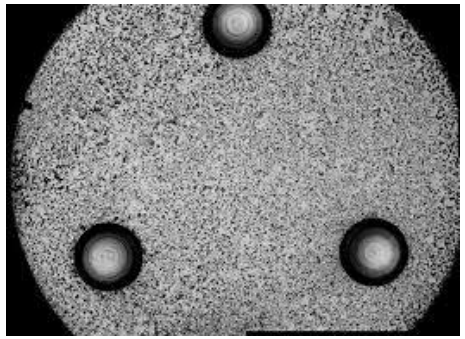


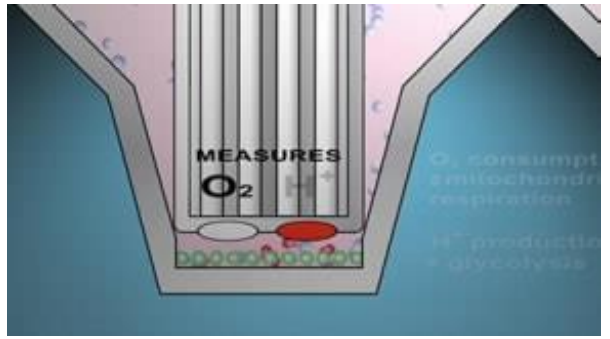
sensor cartridge

sensor

calibration plate

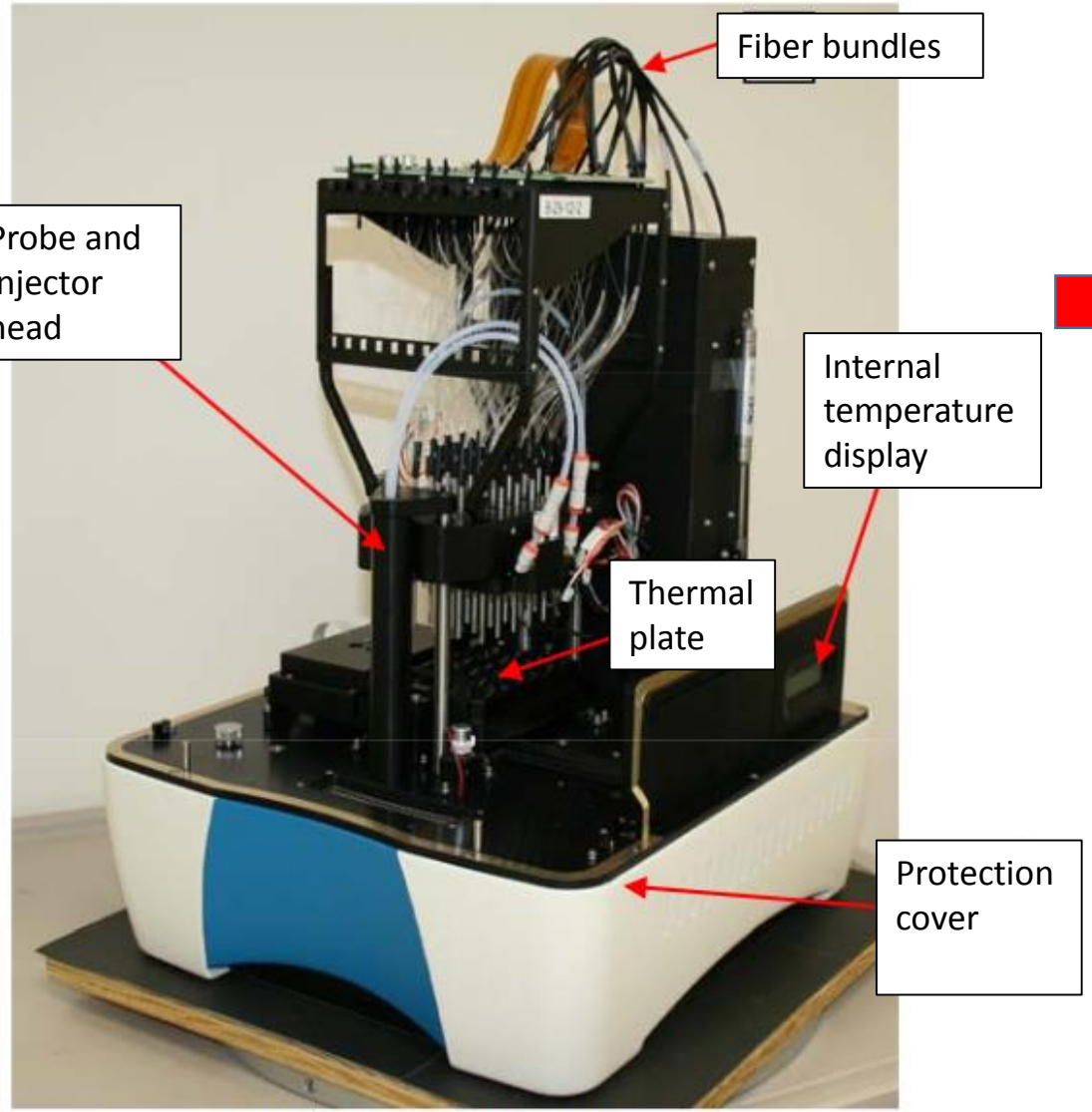






Measurement of O2 and mpH variations
in a small volume (7µL)

SeaHorse® device

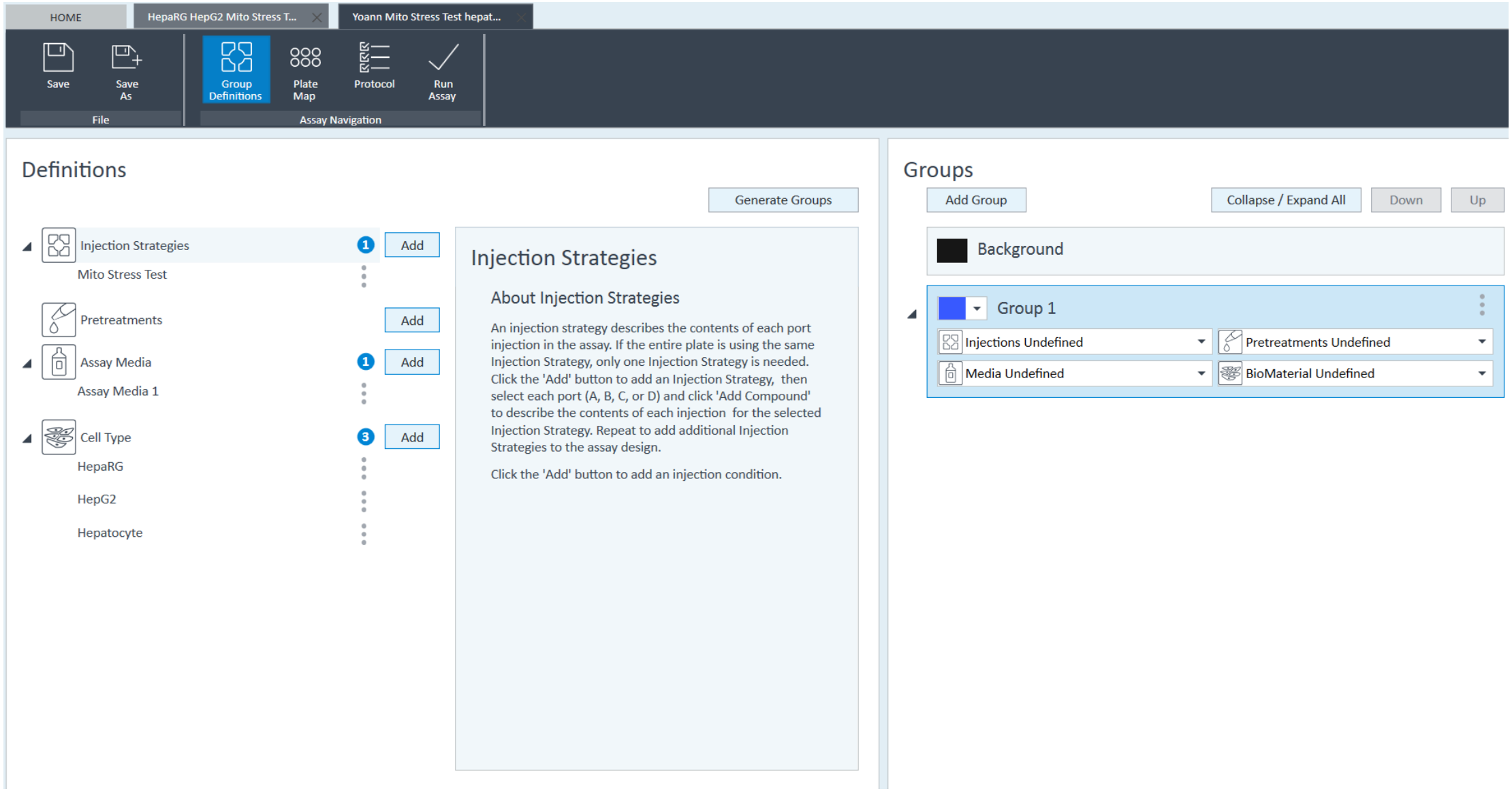


XF^{e} Front/Side View

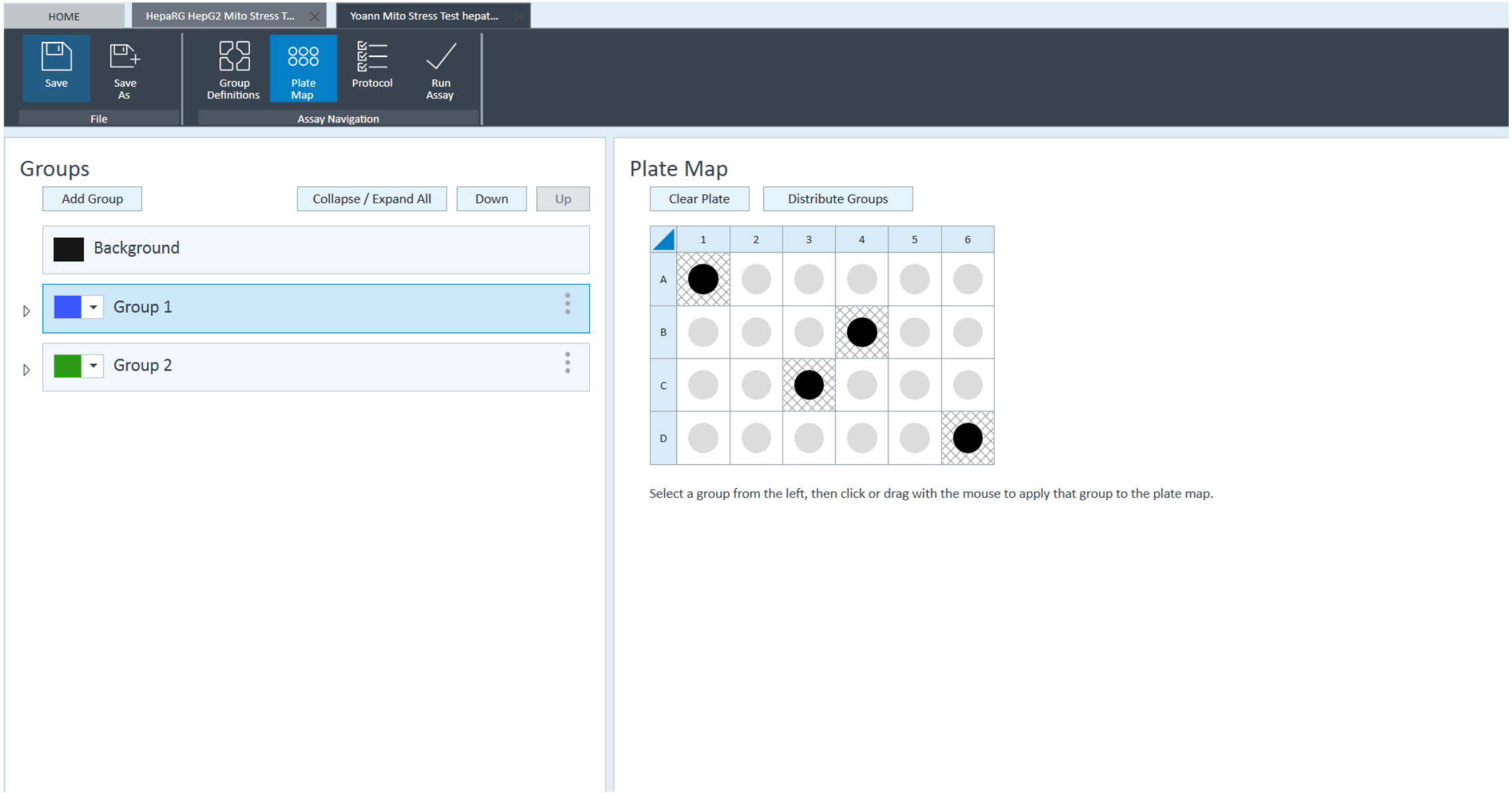
https://www.agilent.com/en/products/cell-analysis/seahorse-analyzers/cell-analysis-measuring-cell-metabolism-transient-micro-chamber

https://www.agilent.com/en/products/cell-analysis/seahorse-analyzers/cell-analysis-seahorse-xf-analyzer-multi-port-drug-injection

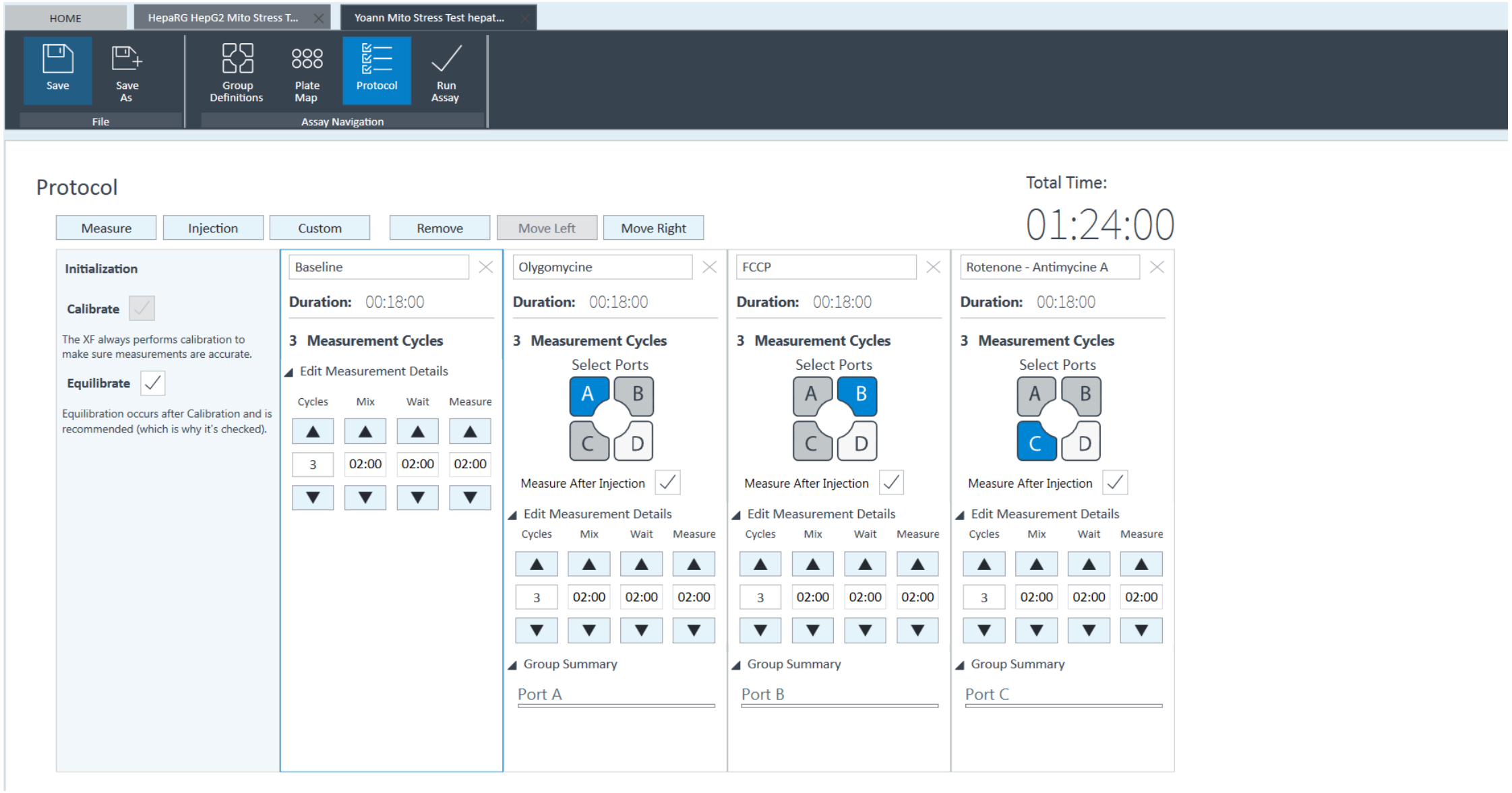
Create a protocol



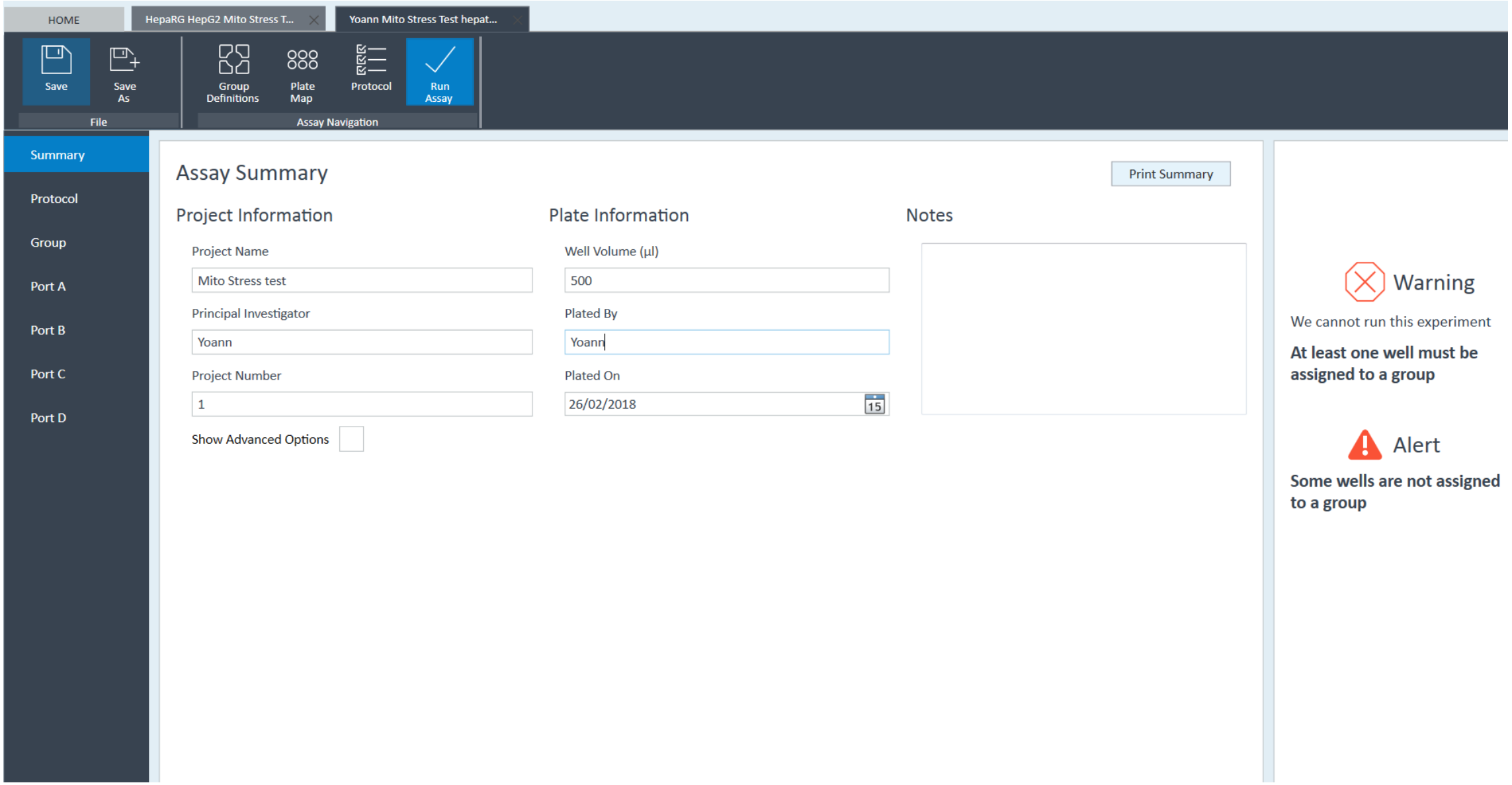
Create a protocol



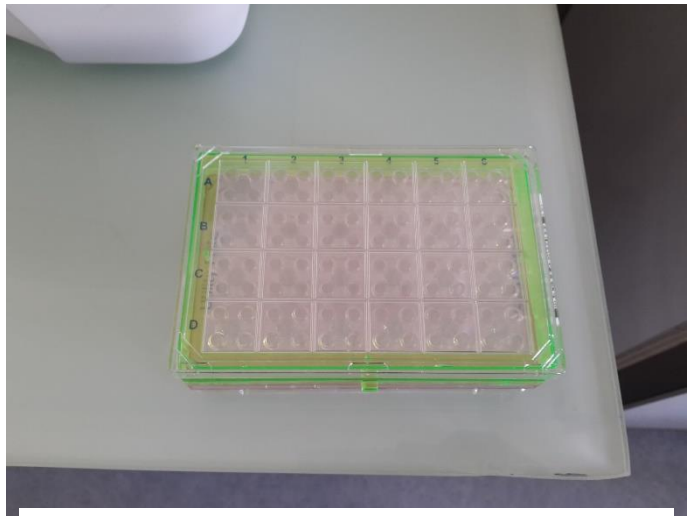
Create a protocol



Create a protocol



Before experiment (Day before)



Hydrate cartridge sensors with XF calibration and place the plate at 37 ° C without CO2 overnight

Prepare the media for the day of the experiment. To do this prepare XF DMEM medium with:

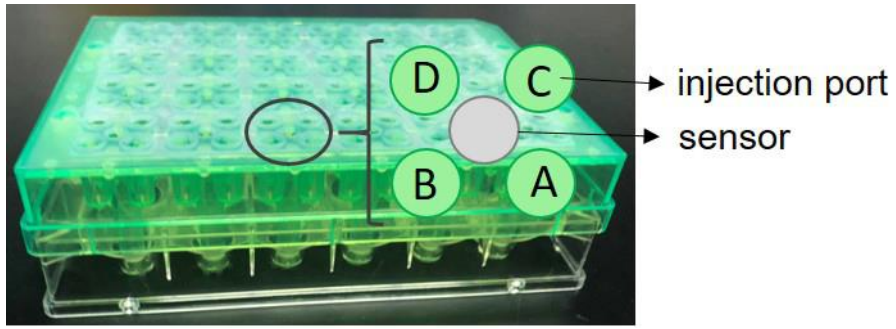
- Glutamine, pyruvate and glucose (Mito Stress Test)
- Glutamine alone (Glycolysis Stress Test)

The pH of the medium must then be adjusted to 7.4

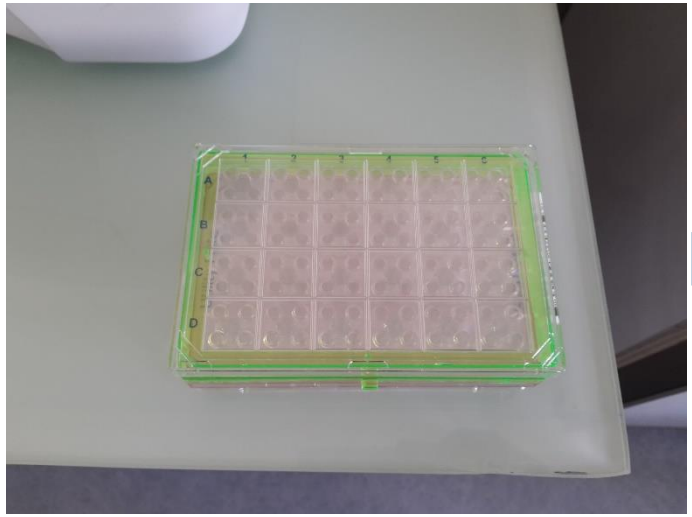
Before launch Seahorse device

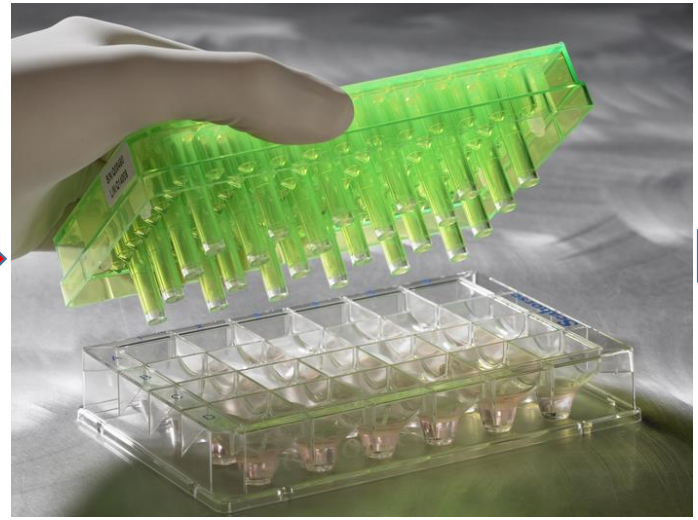
Aspirate the supernatant from the wells and wash with the medium appropriate for the experiment. Then put the plate in the 37 ° C CO2-free incubator for 45 min to 1 hour

Reconstitute the different molecules with the appropriate medium and load them into the cartridge which was in the incubator at 37 ° C overnight

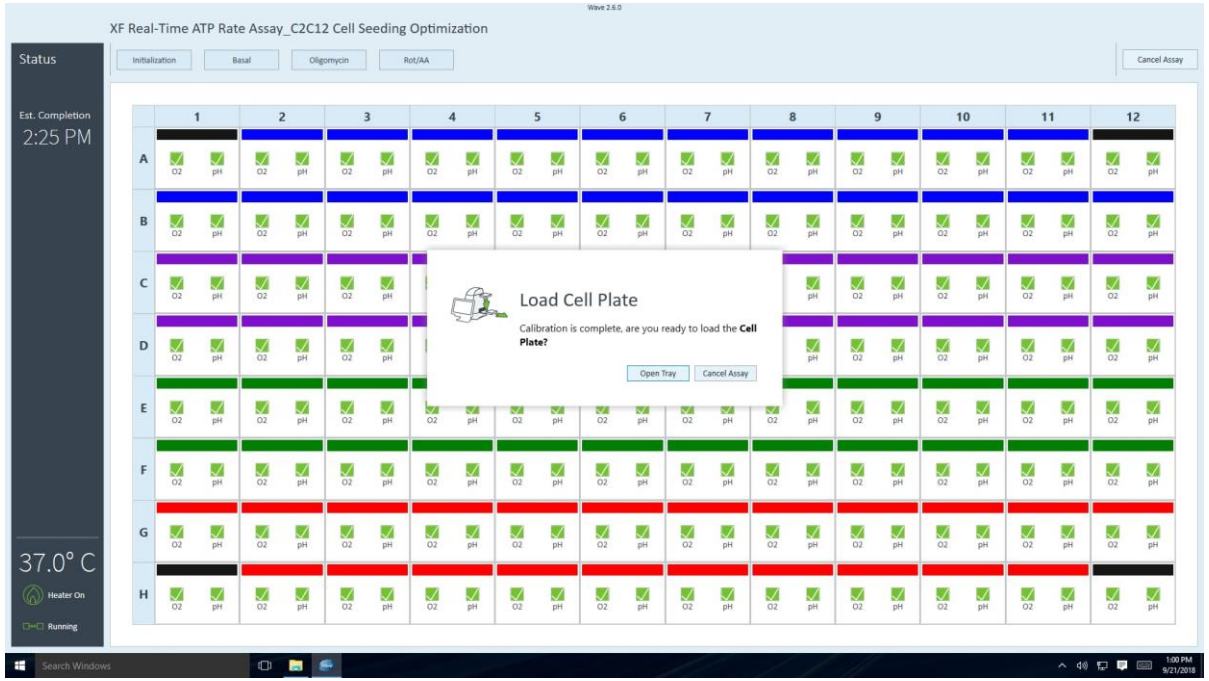


Calibration

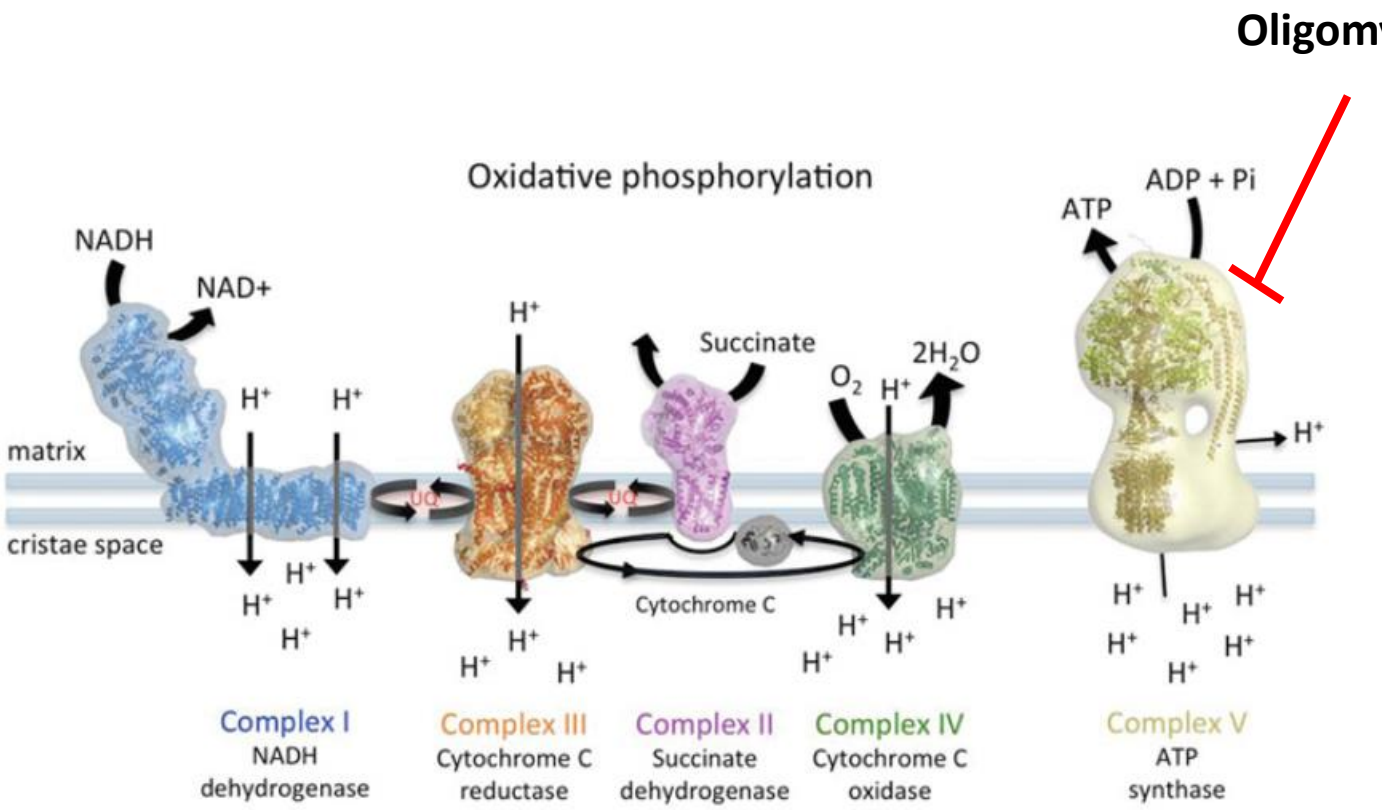


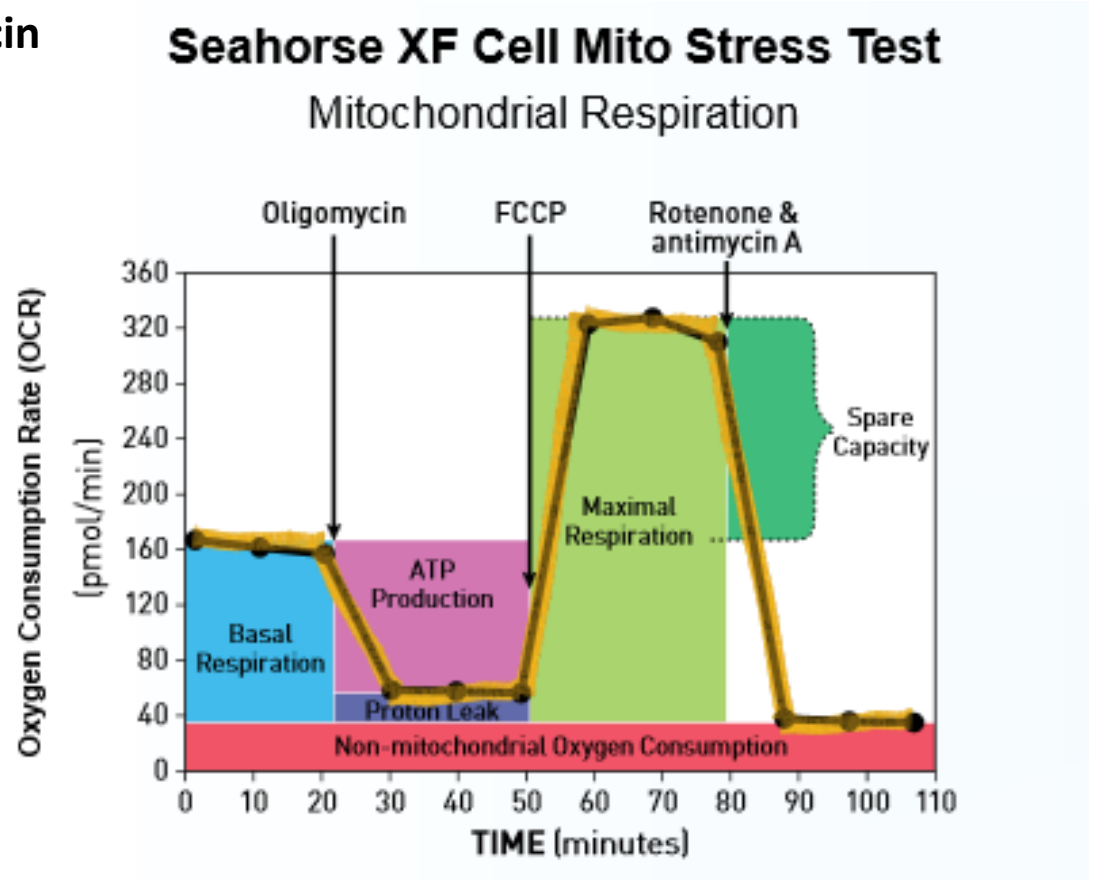


Remove the cover and the pink plate from the cartridge and put it in the device for pH and O2 calibration



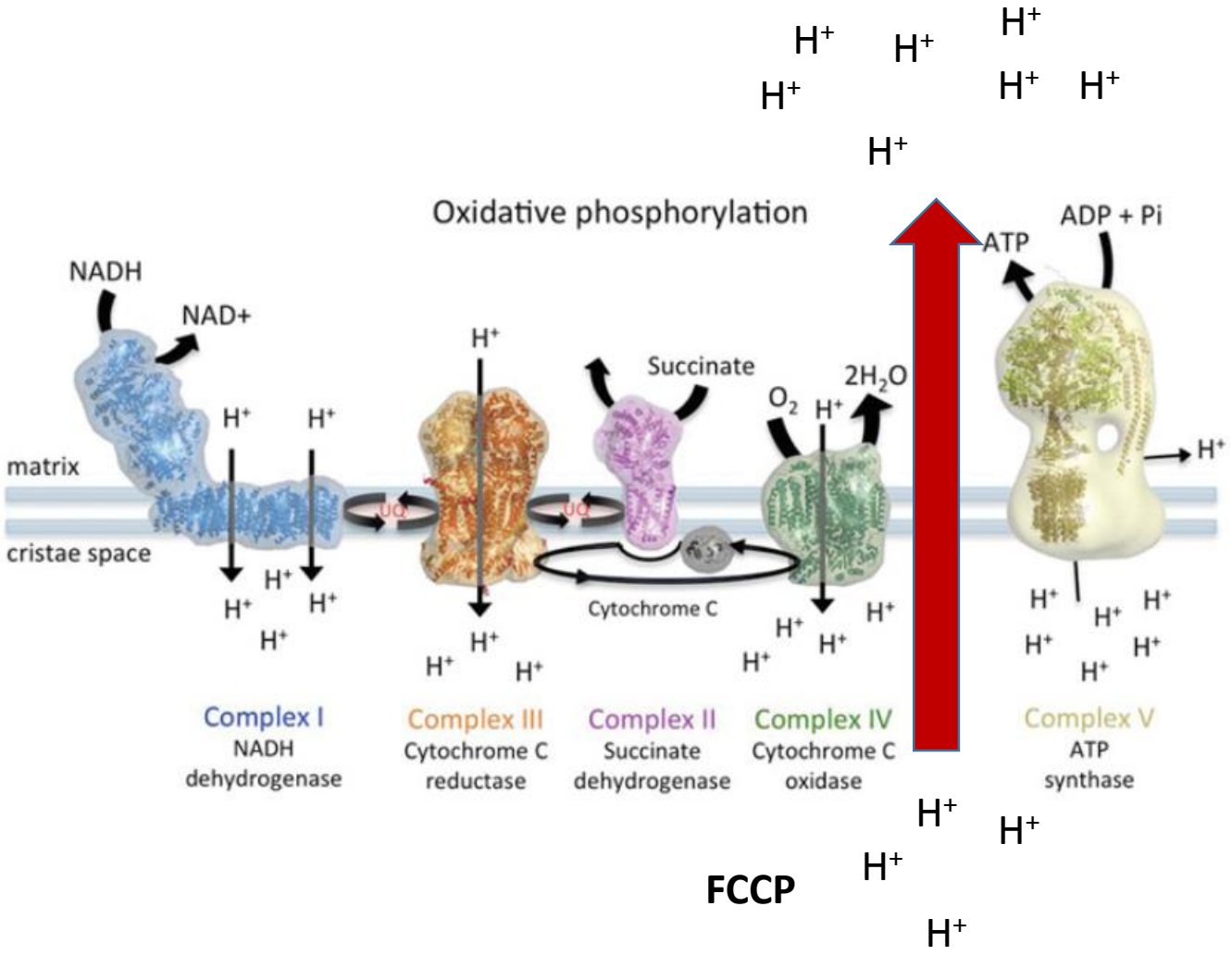
During the run : Mito Stress Test

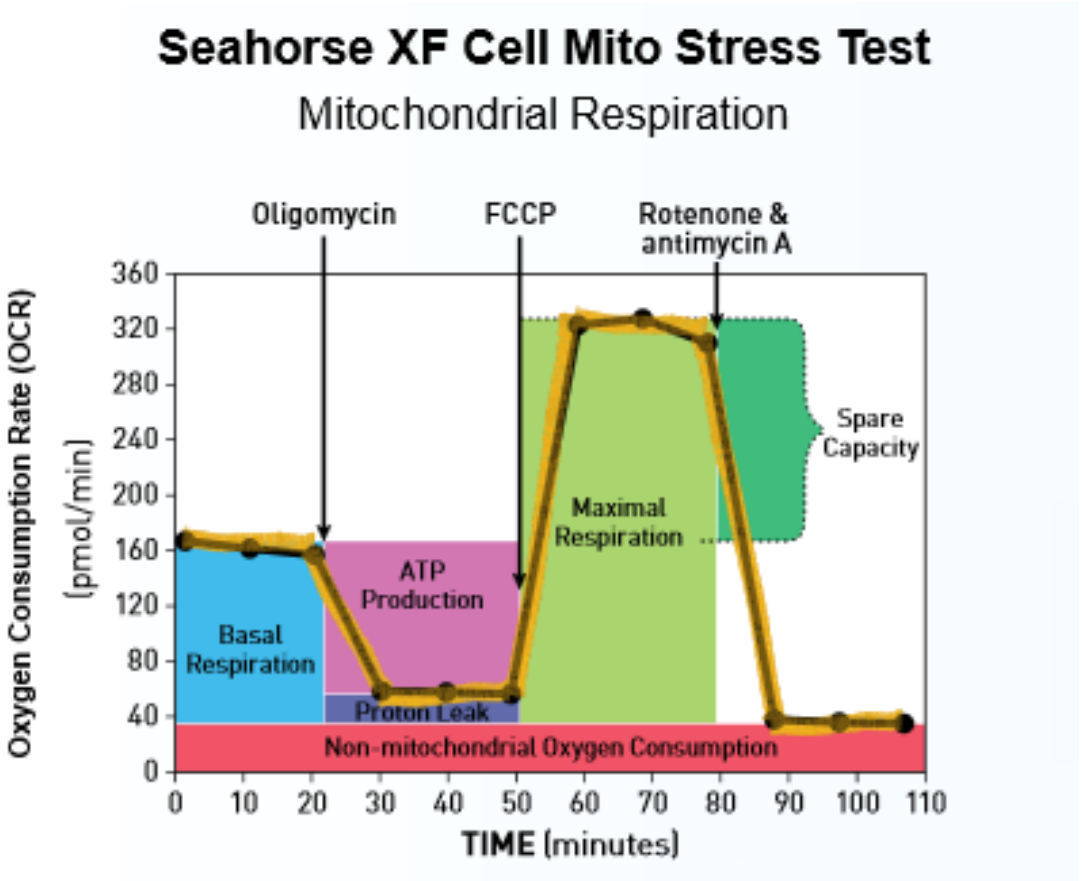




Oligomycin inhibits ATP synthase complex

During the run : Mito Stress Test



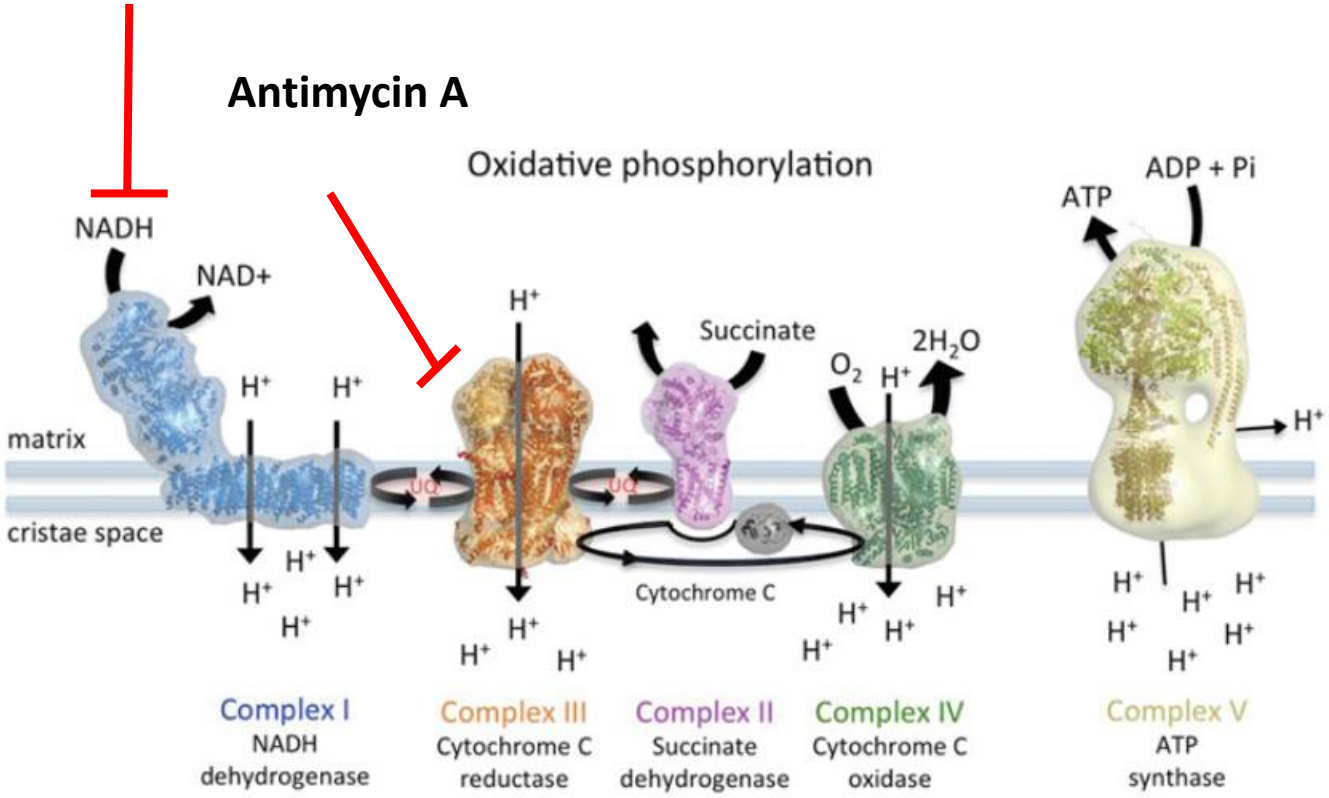


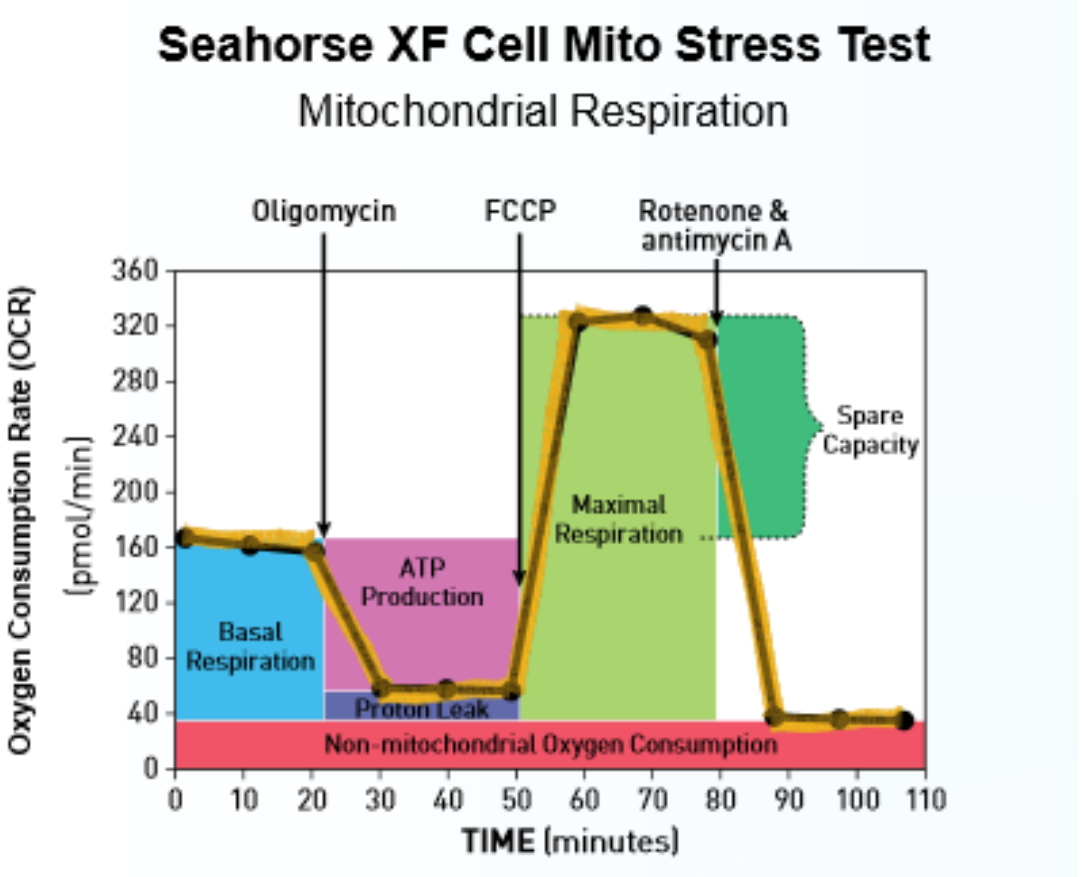
The FCCP will cause a massive entry of protons. The complexes will operate at their maximum capacity

During the run : Mito Stress Test

Rotenone

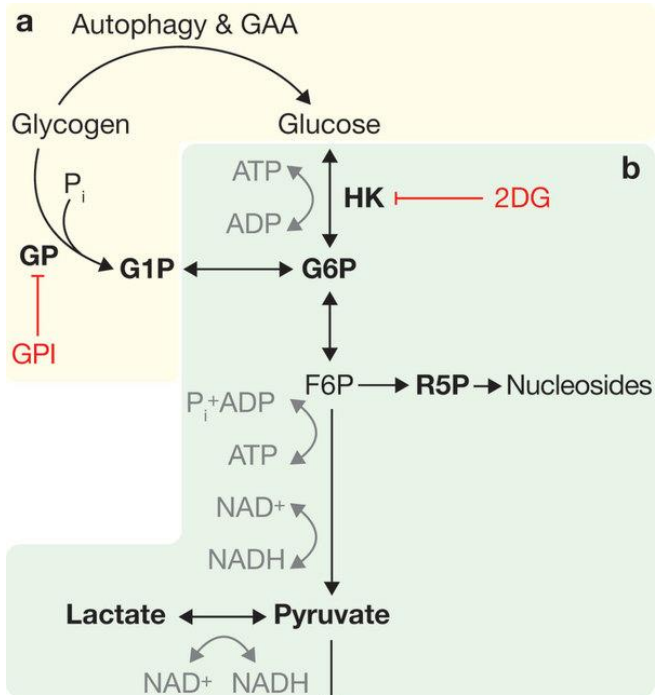
Antimycin A

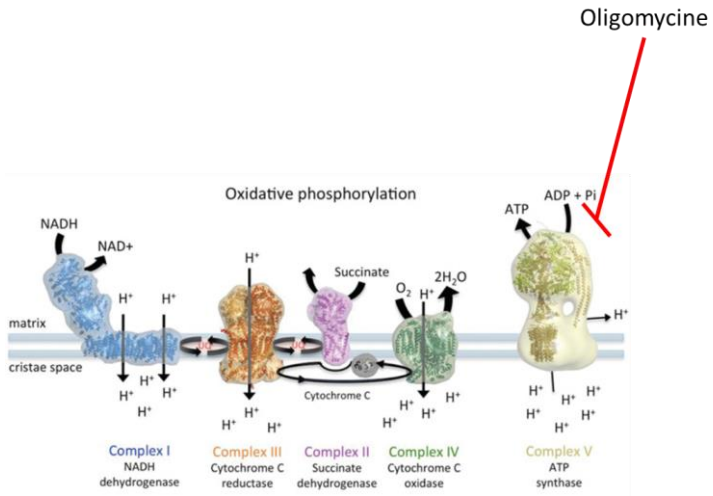


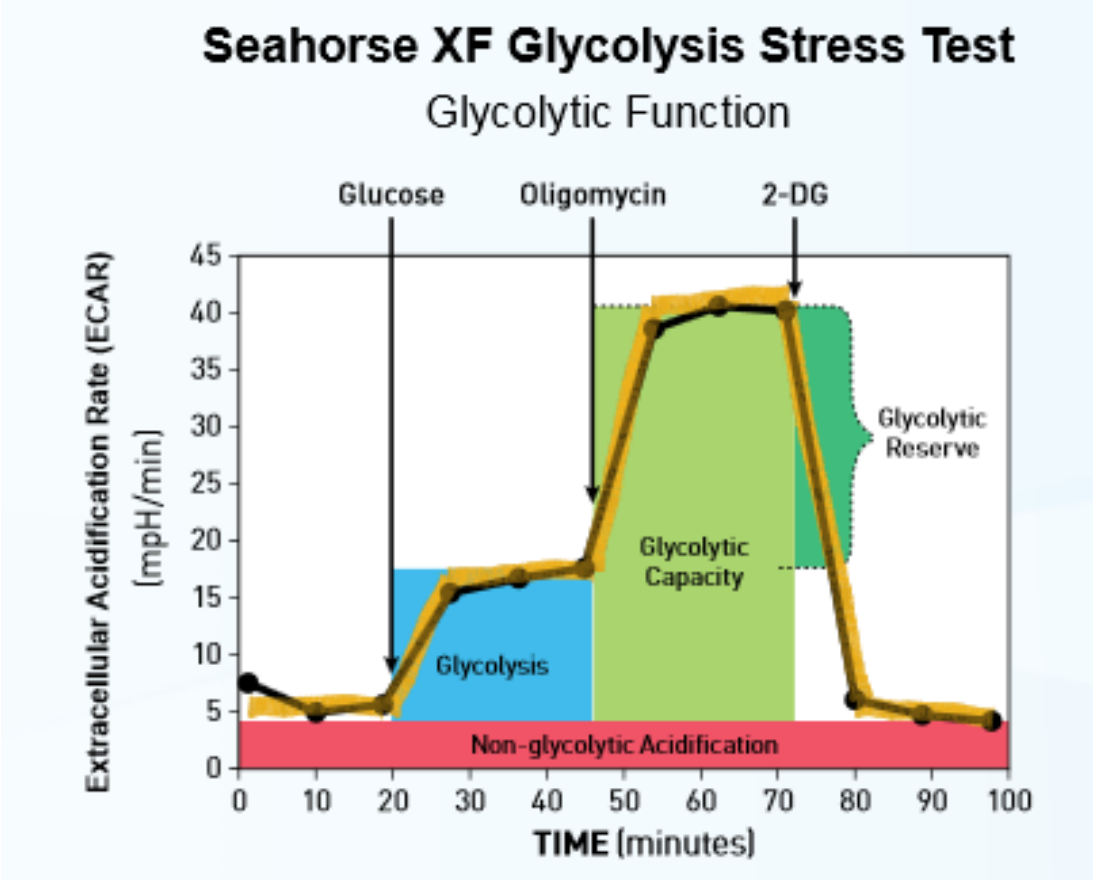


Antimycin A and rotenone inhibit complexes I and III of the respiratory chain causing abrupt arrest of mitochondrial respiration = allows to subtract non-mitochondrial oxygen consumption from all other parameters

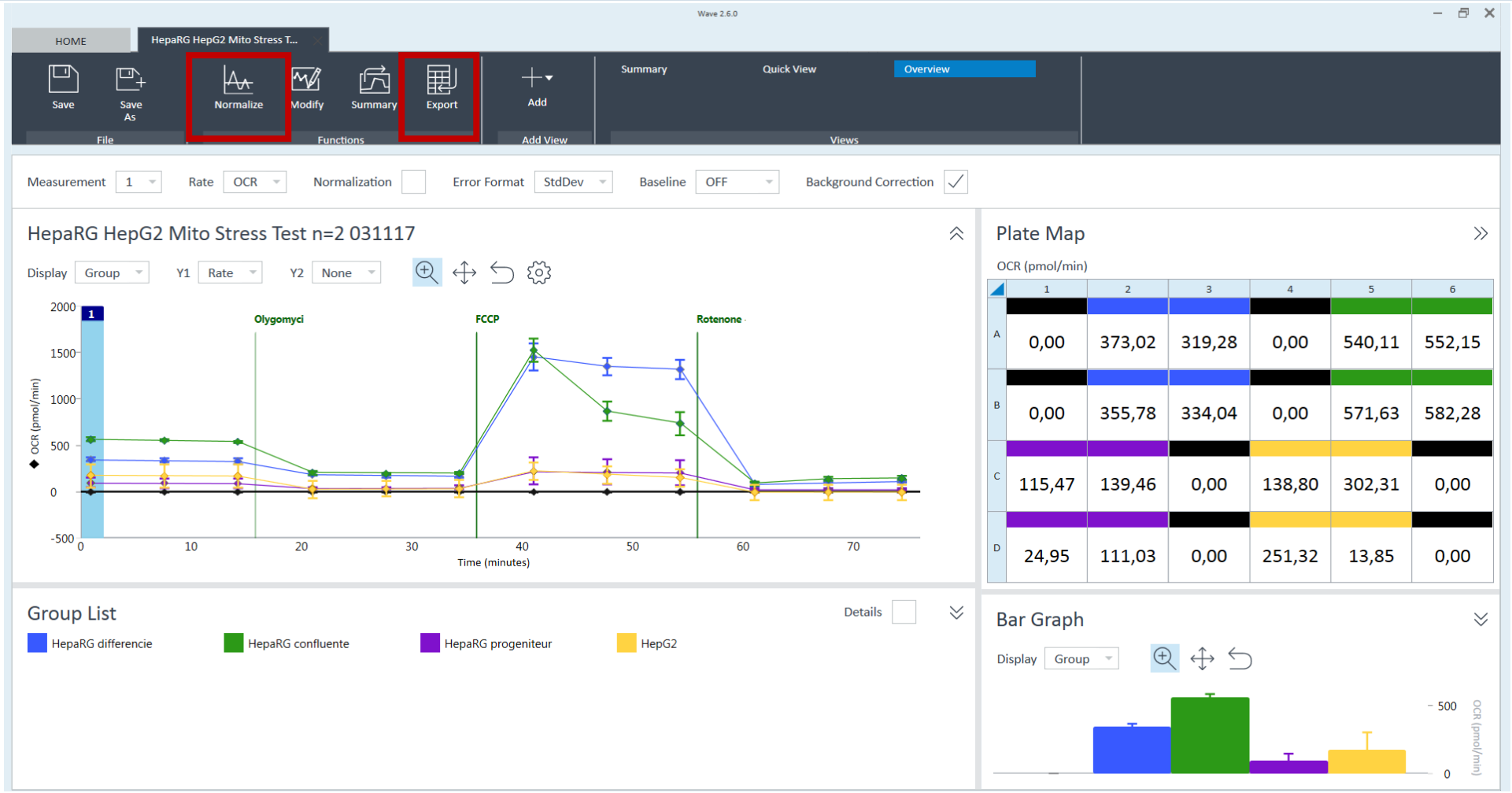
Other example of kit : Glycolysis Stress Test





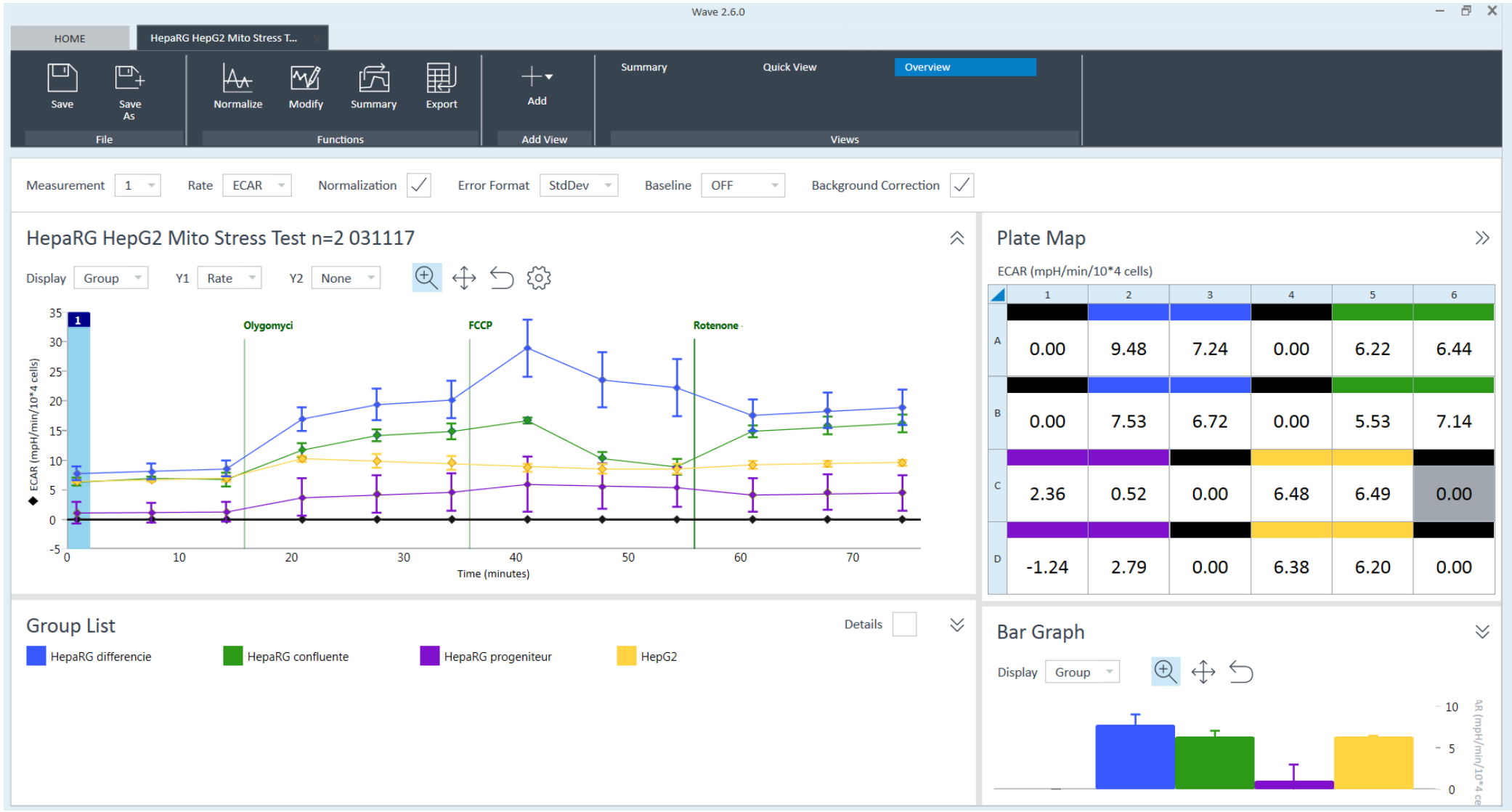


Seahorse® Result with Wave software

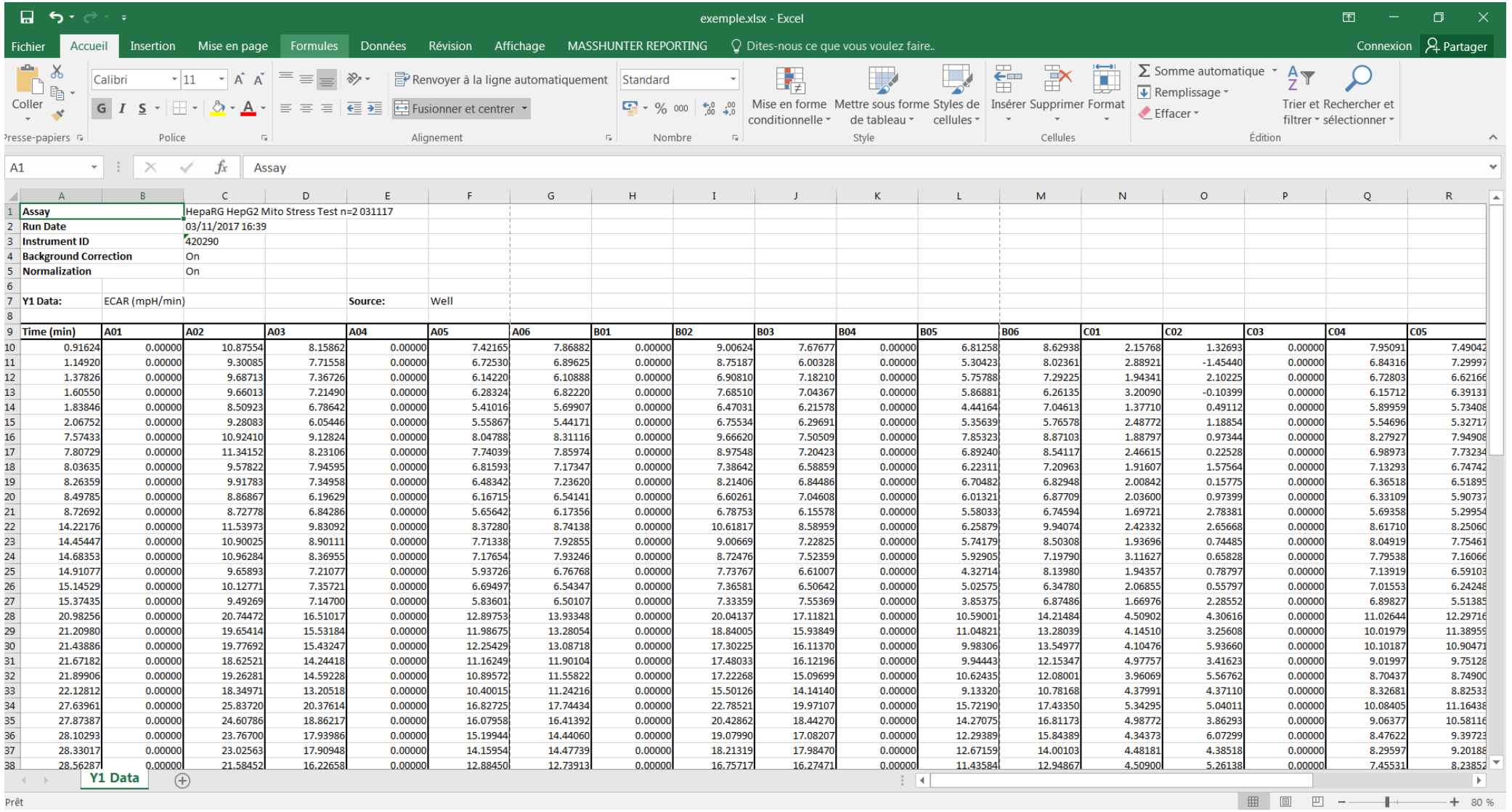


The interface with respiration profiles. We can normalize dataset (protein, cell number…) and export them in an Excel

Seahorse® Result



Seahorse® Result



exemple.xlsx - Excel

	A	B	C	D	E	F
1	**Assay**		HepaRG HepG2 Mito Stress Test n=2 031117			
2	**Run Date**		03/11/2017 16:39			
3	**Instrument ID**		420290			
4	**Background Correction**		On			
5	**Normalization**		On			
6						
7	**Y1 Data:**	ECAR (mpH/min)			**Source:**	Well

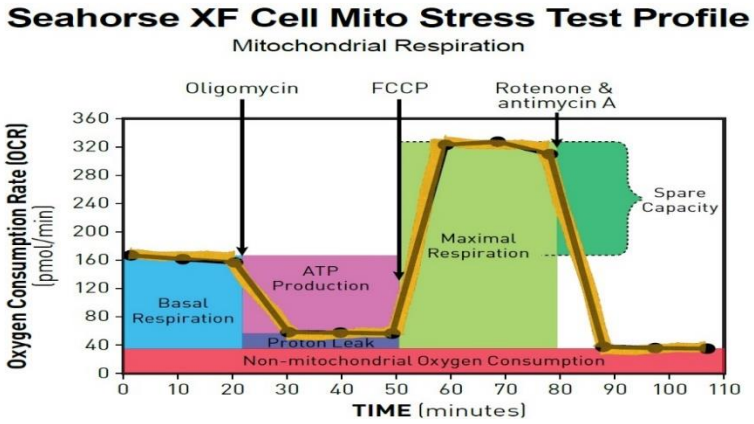
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
9	**Time (min)**	**A01**	**A02**	**A03**	**A04**	**A05**	**A06**	**B01**	**B02**	**B03**	**B04**	**B05**	**B06**	**C01**	**C02**	**C03**	**C04**	**C05**
10	0.91624	0.00000	10.87554	8.15862	0.00000	7.42165	7.86882	0.00000	9.00624	7.67677	0.00000	6.81258	8.62938	2.15768	1.32693	0.00000	7.95091	7.49042
11	1.14920	0.00000	9.30085	7.71558	0.00000	6.72530	6.89625	0.00000	8.75187	6.00328	0.00000	5.30423	8.02361	2.88921	-1.45440	0.00000	6.84316	7.29997
12	1.37826	0.00000	9.68713	7.36726	0.00000	6.14220	6.10888	0.00000	6.90810	7.18210	0.00000	5.75788	7.29225	1.94341	2.10225	0.00000	6.72803	6.62166
13	1.60550	0.00000	9.66013	7.21490	0.00000	6.28324	6.82220	0.00000	7.68510	7.04367	0.00000	5.86881	6.26135	3.20090	-0.10399	0.00000	6.15712	6.39131
14	1.83846	0.00000	8.50923	6.78642	0.00000	5.41016	5.69907	0.00000	6.47031	6.21578	0.00000	4.44164	7.04613	1.37710	0.49112	0.00000	5.89959	5.73408
15	2.06752	0.00000	9.28083	6.05446	0.00000	5.55867	5.44171	0.00000	6.75534	6.29691	0.00000	5.35639	5.76578	2.48772	1.18854	0.00000	5.54696	5.32717
16	7.57433	0.00000	10.92410	9.12824	0.00000	8.04788	8.31116	0.00000	9.66620	7.50509	0.00000	7.85323	8.87103	1.88797	0.97344	0.00000	8.27927	7.94908
17	7.80729	0.00000	11.34152	8.23106	0.00000	7.74039	7.85974	0.00000	8.97548	7.20423	0.00000	6.89240	8.54117	2.46615	0.22528	0.00000	6.98973	7.73234
18	8.03635	0.00000	9.57822	7.94595	0.00000	6.81593	7.17347	0.00000	7.38642	6.58859	0.00000	6.22311	7.20963	1.91607	1.57564	0.00000	7.13293	6.74742
19	8.26359	0.00000	9.91783	7.34958	0.00000	6.48342	7.23620	0.00000	8.21406	6.84486	0.00000	6.70482	6.82948	2.00842	0.15775	0.00000	6.36518	6.51895
20	8.49785	0.00000	8.86867	6.19629	0.00000	6.16715	6.54141	0.00000	6.60261	7.04608	0.00000	6.01321	6.87709	2.03600	0.97399	0.00000	6.33109	5.90737
21	8.72692	0.00000	8.72778	6.84286	0.00000	5.65642	6.17356	0.00000	6.78753	6.15578	0.00000	5.58033	6.74594	1.69721	2.78381	0.00000	5.69358	5.29954
22	14.22176	0.00000	11.53973	9.83092	0.00000	8.37280	8.74138	0.00000	10.61817	8.58959	0.00000	6.25879	9.94074	2.42332	2.65668	0.00000	8.61710	8.25060
23	14.45447	0.00000	10.90025	8.90111	0.00000	7.71338	7.92855	0.00000	9.00669	7.22825	0.00000	5.74179	8.50308	1.93696	0.74485	0.00000	8.04919	7.75461
24	14.68353	0.00000	10.96284	8.36955	0.00000	7.17654	7.93246	0.00000	8.72476	7.52359	0.00000	5.92905	7.19790	3.11627	0.65828	0.00000	7.79538	7.16066
25	14.91077	0.00000	9.65893	7.21077	0.00000	5.93726	6.76768	0.00000	7.73767	6.61007	0.00000	4.32714	8.13980	1.94357	0.78797	0.00000	7.13919	6.59103
26	15.14529	0.00000	10.12771	7.35721	0.00000	6.69497	6.54347	0.00000	7.36581	6.50642	0.00000	5.02575	6.34780	2.06855	0.55797	0.00000	7.01553	6.24248
27	15.37435	0.00000	9.49269	7.14700	0.00000	5.83601	6.50107	0.00000	7.33359	7.55369	0.00000	3.85375	6.87486	1.66976	2.28552	0.00000	6.89827	5.51385
28	20.98256	0.00000	20.74472	16.51017	0.00000	12.89753	13.93348	0.00000	20.04137	17.11821	0.00000	10.59001	14.21484	4.50902	4.30616	0.00000	11.02644	12.29716
29	21.20980	0.00000	19.65414	15.53184	0.00000	11.98675	13.28054	0.00000	18.84005	15.93849	0.00000	11.04821	13.28039	4.14510	3.25608	0.00000	10.01979	11.38955
30	21.43886	0.00000	19.77692	15.43247	0.00000	12.25429	13.08718	0.00000	17.30225	16.11370	0.00000	9.98306	13.54977	4.10476	5.93660	0.00000	10.10187	10.90471
31	21.67182	0.00000	18.62521	14.24418	0.00000	11.16249	11.90104	0.00000	17.48033	16.12196	0.00000	9.94443	12.15347	4.97757	3.41623	0.00000	9.01997	9.75128
32	21.89906	0.00000	19.26281	14.59228	0.00000	10.89572	11.55822	0.00000	17.22268	15.09699	0.00000	10.62435	12.08001	3.96069	5.56762	0.00000	8.70437	8.74900
33	22.12812	0.00000	18.34971	13.20518	0.00000	10.40015	11.24216	0.00000	15.50126	14.14140	0.00000	9.13320	10.78168	4.37991	4.37110	0.00000	8.32681	8.82533
34	27.63961	0.00000	25.83720	20.37614	0.00000	16.82725	17.74434	0.00000	22.78521	19.97107	0.00000	15.72190	17.43350	5.34295	5.04011	0.00000	10.08405	11.16438
35	27.87387	0.00000	24.60786	18.86217	0.00000	16.07958	16.41392	0.00000	20.42862	18.44270	0.00000	14.27075	16.81173	4.98772	3.86293	0.00000	9.06377	10.58116
36	28.10293	0.00000	23.76700	17.93986	0.00000	15.19944	14.44060	0.00000	19.07990	17.08207	0.00000	12.29389	15.84389	4.34373	6.07299	0.00000	8.47622	9.39723
37	28.33017	0.00000	23.02563	17.90948	0.00000	14.15954	14.47739	0.00000	18.21319	17.98470	0.00000	12.67159	14.00103	4.48181	4.38518	0.00000	8.29597	9.20188
38	28.56287	0.00000	21.58452	16.22658	0.00000	12.88450	12.73913	0.00000	16.75717	16.27471	0.00000	11.43584	12.94867	4.50900	5.26138	0.00000	7.45531	8.23852

Y1 Data

Prêt

80 %

Seahorse® Result



Parameter Value	Equation
Non-mitochondrial Oxygen Consumption	Minimum rate measurement after Rotenone/antimycin A injection
Basal Respiration	(Last rate measurement before first injection) – (Non-Mitochondrial Respiration Rate)
Maximal Respiration	(Maximum rate measurement after FCCP injection) – (Non-Mitochondrial Respiration)
H+ (Proton) Leak	(Minimum rate measurement after Oligomycin injection) – (Non-Mitochondrial Respiration
ATP Production	(Last rate measurement before Oligomycin injection) – (Minimum rate measurement after Oligomycin injection)
Spare Respiratory Capacity	(Maximal Respiration) – (Basal Respiration)
Spare Respiratory Capacity as a %	(Maximal Respiration) / (Basal Respiration) × 100
Acute Response	(Last rate measurement before oligomycin Injection) – (Last rate measurement before acute injection)
Coupling Efficiency	ATP Production Rate) / (Basal Respiration Rate) × 100

Experimental Group #1							
	Measurement	Assay Well - A1 *OCR (pmol/min)*	Assay Well - A2 *OCR (pmol/min)*	Assay Well - A3 *OCR (pmol/min)*	Assay Well - A4 *OCR (pmol/min)*	Average OCR	
Baseline OCR	1	170.02	172.80	169.96	175.99	172.19	
	2	165.36	163.62	167.00	166.42	165.60	
	3	160.50	158.25	159.44	161.75	159.98	<< Last rate measurement before 1st injection
Injection 1 - Oligomycin	4	65.44	53.05	61.53	59.22	59.81	
	5	61.80	49.44	59.62	56.94	56.95	
	6	61.93	49.55	59.06	56.04	56.65	<< Minimum rate measurement after Oligo injection
Injection 2 - FCCP	7	319.58	310.94	312.59	315.98	314.77	
	8	327.95	320.32	325.77	330.73	326.19	<< Maximum rate measurement after FCCP Injection
	9	297.17	292.30	301.35	299.68	297.63	
Injection 3 - Rot/AA	10	51.77	34.62	44.99	39.45	42.71	
	11	49.28	33.24	44.13	39.05	41.42	
	12	47.03	31.80	42.66	39.20	40.17	<< Minimum after Rot/AA Injection (Non-Mitochondrial Oxygen Consumption)

Individual OCR Values for Assay Wells					
	Measurement	A1	A2	A3	A4
Baseline OCR	3	160.50	158.25	169.44	161.75
Injection 1 - Oligomycin	6	61.93	49.55	59.06	56.04
Injection 2 - FCCP	8	327.95	320.32	325.77	330.73
Injection 3 - Rot/AA	12	47.03	31.80	42.66	39.20

Parameter Calculations *(per well)*	Assay Wells				Displayed Values	
	A1	A2	A3	A4	Average	StDev
Non-Mitochondrial Oxygen Consumption	47.03	31.80	42.66	39.20	**40.17**	**6.43**
Basal Respiration	113.47	126.45	126.78	122.55	**122.31**	**6.20**
Maximum Respiration	280.92	288.52	283.11	291.53	**286.02**	**4.87**
H+ (Proton) Leak	14.91	17.75	16.40	16.85	**16.48**	**1.19**
ATP Production	98.57	108.70	110.38	105.71	**105.84**	**5.22**
Spare Respiratory Capacity	167.45	162.07	156.33	168.98	**163.71**	**5.74**

Mito Stress Test : interpretation

Basal respiration: Basal oxygen consumption rate (OCR) is the OCR or the rate at which mitochondria function in a cell type under the conditions that you have provided in the culture

ATP production: Oligomycin sensitive or ATP-dependent OCR is the OCR required to synthesis ATP only at complex V in the mitochondria

Proton leak: This coupling of ATP synthesis and substrate oxidation is not complete, as protons can return to the matrix independently of ATP synthase. The processes by which this occurs are collectively termed “proton leak”

Spare capacity: Spare respiratory capacity or reserve capacity is the difference between maximum OCR after treating the cell with an ionophore and basal OCR, which is an estimate of the potential bioenergetic reserve the cell can call upon in times of stress

Non mitochondrial OCR: This parameter is an index of oxygen consuming processes which are not mitochondrial. In leukocytes, non-mitochondrial OCR is typically attributed to enzymes associated with inflammation, including cyclooxygenases, lipooxygenases and NADPH oxidases, and regarded as negative indicators of bioenergetic health. Non-mitochondrial OCR varies, and typically increases in the presence of stressors, including ROS and RNS and it is well established that mitochondria are a target for the deleterious effects of these reactive intermediates.

Agilent website

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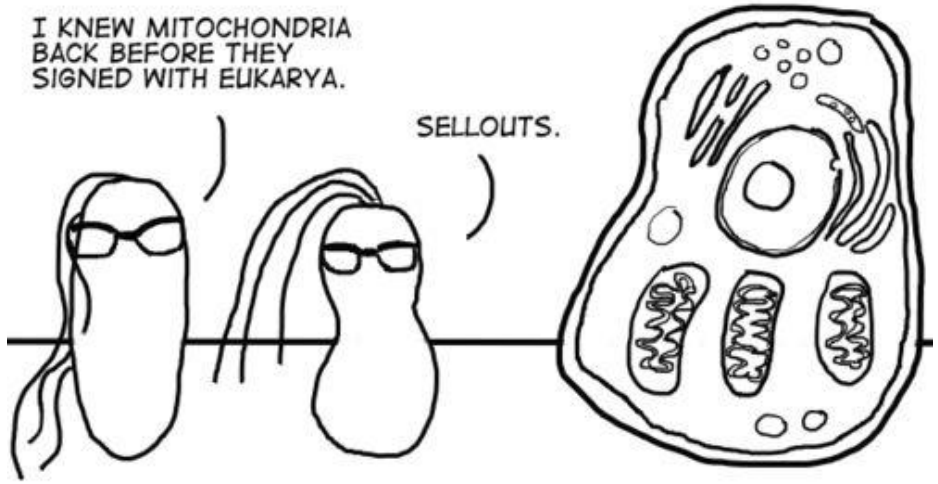
Thank you for your attention !



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