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Generating Antiviral & Cytotoxicity Curves

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We use this protocol and it's working

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Abstract

This describes generating Antiviral & Cytotoxicity Curves

Analyzing the Cytotoxicity Plates

1 After you scan the plates and export the data to excel you will have an output that looks like this:
If you used the MTS kit you will only have one
If you used the Roche MTT kit you will have two readings

								Nirmatrelvir					
	1	2	3	4	5	6	7	8	9	10	11	12	
A	0.555	0.233	0.295	0.275	0.373	0.337	0.349	0.668	0.763	0.821	0.815	0.181	490
B	0.696	0.716	0.974	0.924	0.985	0.906	0.937	0.979	1.021	0.832	0.862	0.176	490
C	0.618	0.99	1.112	1.186	1.238	1.113	1.225	1.151	1.123	1.159	1.303	0.175	490
D	0.594	0.938	1.066	1.151	1.193	1.014	0.902	0.973	1.224	1.101	0.946	0.175	490
E	0.552	0.887	1.146	1.14	1.055	0.933	1.177	0.922	1.036	1.225	1.156	0.169	490
F	0.661	0.785	1.113	1.099	1.157	0.946	1.024	1.044	1.138	1.171	1.214	0.169	490
G	0.568	0.833	1.003	0.938	1.127	0.934	1.134	0.854	1.169	1.208	0.998	0.189	490
H	0.548	0.547	0.506	0.537	0.588	0.612	0.94	0.854	0.872	0.837	0.922	0.188	490

MTS plate data

As a reminder, this is the typical layout of the plates:

		DMSO	drug 1			drug 2			drug 3			DMSO	uninfected
		1	2	3	4	5	6	7	8	9	10	11	12
[maximum]	A												
	B												
	C												
	D												
	E												
	F												
	G												
[minimum]	H												

Typical plate layout.

1.1 If you used the MTT kit you will first subtract the values from the two read outs:

2 First, take calculate the average value and standard deviation of columns 1 and 11. These are the DMSO controls.

								Nirmatrelvir					
	1	2	3	4	5	6	7	8	9	10	11	12	
A	0.555	0.233	0.295	0.275	0.373	0.337	0.349	0.668	0.763	0.821	0.815	0.181	490
B	0.696	0.716	0.974	0.924	0.985	0.906	0.937	0.979	1.021	0.832	0.862	0.176	490
C	0.618	0.99	1.112	1.186	1.238	1.113	1.225	1.151	1.123	1.159	1.303	0.175	490
D	0.594	0.938	1.066	1.151	1.193	1.014	0.902	0.973	1.224	1.101	0.946	0.175	490
E	0.552	0.887	1.146	1.14	1.055	0.933	1.177	0.922	1.036	1.225	1.156	0.169	490
F	0.661	0.785	1.113	1.099	1.157	0.946	1.024	1.044	1.138	1.171	1.214	0.169	490
G	0.568	0.833	1.003	0.938	1.127	0.934	1.134	0.854	1.169	1.208	0.998	0.189	490
H	0.548	0.547	0.506	0.537	0.588	0.612	0.94	0.854	0.872	0.837	0.922	0.188	490

DMSO controls columns 1 and 11 highlighted.

3 Next, you will take the average of the three replicates for each dilution of the compounds.

	Nirmatrelvir												
	1	2	3	4	5	6	7	8	9	10	11	12	
A	0.555	0.233	0.295	0.275	0.373	0.337	0.349	0.668	0.763	0.821	0.815	0.181	490
B	0.696	0.716	0.974	0.924	0.985	0.906	0.937	0.979	1.021	0.832	0.862	0.176	490
C	0.618	0.99	1.112	1.186	1.238	1.113	1.225	1.151	1.123	1.159	1.303	0.175	490
D	0.594	0.938	1.066	1.151	1.193	1.014	0.902	0.973	1.224	1.101	0.946	0.175	490
E	0.552	0.887	1.146	1.14	1.055	0.933	1.177	0.922	1.036	1.225	1.156	0.169	490
F	0.661	0.785	1.113	1.099	1.157	0.946	1.024	1.044	1.138	1.171	1.214	0.169	490
G	0.568	0.833	1.003	0.938	1.127	0.934	1.134	0.854	1.169	1.208	0.998	0.189	490
H	0.548	0.547	0.506	0.537	0.588	0.612	0.94	0.854	0.872	0.837	0.922	0.188	490

The triplicates of the first dilution of drug 1 on this plate.

	Nirmatrelvir												
	1	2	3	4	5	6	7	8	9	10	11	12	
A	0.555	0.233	0.295	0.275	0.373	0.337	0.349	0.668	0.763	0.821	0.815	0.181	490
B	0.696	0.716	0.974	0.924	0.985	0.906	0.937	0.979	1.021	0.832	0.862	0.176	490
C	0.618	0.99	1.112	1.186	1.238	1.113	1.225	1.151	1.123	1.159	1.303	0.175	490
D	0.594	0.938	1.066	1.151	1.193	1.014	0.902	0.973	1.224	1.101	0.946	0.175	490
E	0.552	0.887	1.146	1.14	1.055	0.933	1.177	0.922	1.036	1.225	1.156	0.169	490
F	0.661	0.785	1.113	1.099	1.157	0.946	1.024	1.044	1.138	1.171	1.214	0.169	490
G	0.568	0.833	1.003	0.938	1.127	0.934	1.134	0.854	1.169	1.208	0.998	0.189	490
H	0.548	0.547	0.506	0.537	0.588	0.612	0.94	0.854	0.872	0.837	0.922	0.188	490

The triplicates of the second dilution of drug 1 on this plate.

4 Next, you will divide the averages and standard deviations of each dilution by those of the DMSO controls and turn it into a percentage.

For simplicity in excel you can combine steps 3 and 4 into one formula:
=(average(A2-A4) / average(columns 1 & 11)) *100
=(standard deviation(A2-A4) / standard deviation(columns 1 & 11)) *100

				Nirmatrelvir	
% Viability	**STDV**	**% Viability**	**STDV**	**% Viability**	**STDV**
32.92332923	3.892233	43.4194342	2.25465	92.33292333	9.500866
107.1750718	16.82976	115.949159	4.895902	116.1131611	12.2069
134.8093481	12.17402	146.617466	8.453147	140.7544075	2.325125
129.3562936	13.18831	127.470275	18.05411	135.2193522	15.43768
130.0943009	18.18353	129.766298	15.00615	130.504305	18.82401
122.8782288	22.812	128.208282	13.12276	137.4743747	8.105336
113.7351374	10.55114	130.99631	13.96105	132.4723247	23.87523
65.19065191	2.629466	87.7408774	24.1901	105.0840508	2.152814

Output

5 Plug these values into a Prism file for the cell viability section.

Analyzing the Antiviral Plates

6 After staining and reading the plates you will export the data to an excel sheet. The data set that we want to further analyze is the ratio of infected to uninfected cells.

It should look like this:

Ratio

	Nirmatrelvir												
	1	2	3	4	5	6	7	8	9	10	11	12	
A	27.348	16.832	10.061	9.948	0	0	0.373	0.045	0.046	0	28.184	0.114	Ratio
B	25.363	20.556	19.046	19.952	0.033	0.065	0.033	0.034	0.034	0.037	23.851	0.324	Ratio
C	23.687	22.245	20.886	22.905	0.962	1.504	1.272	0.143	0.556	0.181	24.861	1.012	Ratio
D	23.895	24.304	20.248	20.901	6.333	7.643	6.764	5.881	6.747	6.262	20.809	0.055	Ratio
E	23.732	20.599	19.774	21.158	8.231	11.062	10.623	15.185	7.851	19.551	23.515	0.223	Ratio
F	23.098	21.445	18.617	20.479	16.434	14.685	12.341	19.727	22.286	24.459	23.204	0.057	Ratio
G	23.465	21.581	21.286	20.656	18.724	18.42	19.28	21.85	23.441	25.691	26.135	0.061	Ratio
H	25.656	29.104	26.986	25.916	23.664	23.031	26.392	25.26	26.302	33.191	28.142	0.077	Ratio

Antiviral plate data output. Ratio of infected to uninfected cells.

7 Again, take the average of the infected DMSO controls. Columns 1 and 11.

	Nirmatrelvir												
	1	2	3	4	5	6	7	8	9	10	11	12	
A	27.348	16.832	10.061	9.948	0	0	0.373	0.045	0.046	0	28.184	0.114	Ratio
B	25.363	20.556	19.046	19.952	0.033	0.065	0.033	0.034	0.034	0.037	23.851	0.324	Ratio
C	23.687	22.245	20.886	22.905	0.962	1.504	1.272	0.143	0.556	0.181	24.861	1.012	Ratio
D	23.895	24.304	20.248	20.901	6.333	7.643	6.764	5.881	6.747	6.262	20.809	0.055	Ratio
E	23.732	20.599	19.774	21.158	8.231	11.062	10.623	15.185	7.851	19.551	23.515	0.223	Ratio
F	23.098	21.445	18.617	20.479	16.434	14.685	12.341	19.727	22.286	24.459	23.204	0.057	Ratio
G	23.465	21.581	21.286	20.656	18.724	18.42	19.28	21.85	23.441	25.691	26.135	0.061	Ratio
H	25.656	29.104	26.986	25.916	23.664	23.031	26.392	25.26	26.302	33.191	28.142	0.077	Ratio

infected DMSO controls highlighted.

8 Next, take the average of the uninfected control (column 12)

	Nirmatrelvir												
	1	2	3	4	5	6	7	8	9	10	11	12	
A	27.348	16.832	10.061	9.948	0	0	0.373	0.045	0.046	0	28.184	0.114	Ratio
B	25.363	20.556	19.046	19.952	0.033	0.065	0.033	0.034	0.034	0.037	23.851	0.324	Ratio
C	23.687	22.245	20.886	22.905	0.962	1.504	1.272	0.143	0.556	0.181	24.861	1.012	Ratio
D	23.895	24.304	20.248	20.901	6.333	7.643	6.764	5.881	6.747	6.262	20.809	0.055	Ratio
E	23.732	20.599	19.774	21.158	8.231	11.062	10.623	15.185	7.851	19.551	23.515	0.223	Ratio
F	23.098	21.445	18.617	20.479	16.434	14.685	12.341	19.727	22.286	24.459	23.204	0.057	Ratio
G	23.465	21.581	21.286	20.656	18.724	18.42	19.28	21.85	23.441	25.691	26.135	0.061	Ratio
H	25.656	29.104	26.986	25.916	23.664	23.031	26.392	25.26	26.302	33.191	28.142	0.077	Ratio

uninfected control highlighted

9 then, calculate the following using excel.
[((value of experimental well) - (average uninfected)) / (average infected DMSO controls)]*100

So, from the above example data it will be:
[(2A- value from step 8) / (value from step 7)] *100

[(16.832-0.240375)/(24.684063)] = 67.21594

The data will look like this:

						Nirmatrelvir		
67.215941	39.785287	39.327501	-0.973806	-0.973806	0.53729	-0.791503	-0.787451	-0.973806
82.302599	76.185292	79.855676	-0.840117	-0.710479	-0.840117	-0.836066	-0.836066	-0.823912
89.145071	83.639494	91.818861	2.923445	5.1191938	4.1793161	-0.394485	1.2786591	-0.24054
97.486485	81.05483	83.700262	24.682424	29.989492	26.42849	22.851283	26.35962	24.394789
82.476801	79.134563	84.74142	32.371596	43.840535	42.062059	60.54362	30.832141	78.231146
85.904113	74.447328	81.990657	65.603565	58.518021	49.022016	78.944157	89.31117	98.114421
86.455076	85.259973	82.707719	74.880806	73.649242	77.133272	87.544848	93.990302	103.1055
116.93223	108.3518	104.01702	94.893719	92.329312	105.94538	101.35943	105.58078	133.48947

Analysis of data from this example experiment.

10 From the output of step 9, calculate the average and standard deviation **between the triplicates**

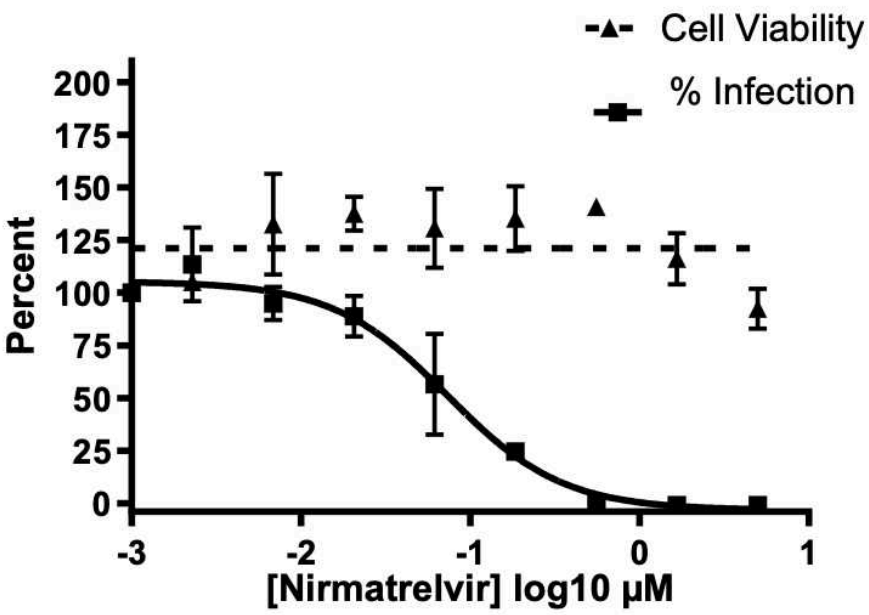
						Nirmatrelvir							Nirmatrelvir	
67.215941	39.785287	39.327501	-0.973806	-0.973806	0.53729	-0.791503	-0.787451	-0.973806		% Infection	STDV	% Infection	STDV	% Infection	STDV
82.302599	76.185292	79.855676	-0.840117	-0.710479	-0.840117	-0.836066	-0.836066	-0.823912		79.447856	3.0789772	-0.796904	0.0748467	-0.832015	0.0070169
89.145071	83.639494	91.818861	2.923445	5.1191938	4.1793161	-0.394485	1.2786591	-0.24054		88.201142	4.1705829	4.073985	1.1016575	0.2145446	0.9247591
97.486485	81.05483	83.700262	24.682424	29.989492	26.42849	22.851283	26.35962	24.394789		87.413859	8.8228638	27.033469	2.7047629	24.535231	1.7583797
82.476801	79.134563	84.74142	32.371596	43.840535	42.062059	60.54362	30.832141	78.231146		82.117594	2.8206351	39.42473	6.172582	56.535636	23.952335
85.904113	74.447328	81.990657	65.603565	58.518021	49.022016	78.944157	89.31117	98.114421		80.780699	5.8234422	57.714534	8.3199242	88.789916	9.5957562
86.455076	85.259973	82.707719	74.880806	73.649242	77.133272	87.544848	93.990302	103.1055		84.807589	1.9141995	75.221107	1.7667678	94.880215	7.8184012
116.93223	108.3518	104.01702	94.893719	92.329312	105.94538	101.35943	105.58078	133.48947		109.76701	6.5728862	97.722805	7.2354791	113.47656	17.459738

Left: Data output from step 9
Right: Average and standard deviation of the triplicates from step 9.

11 Plug these values into a Prism file for the % infection section.

Prism Output

12



Antiviral		Cytotoxicity	
IC50	0.048 uM	CC50	>5 uM
IC90	0.101 uM	CC10	>5 uM

This is what the final Prism output should look like.