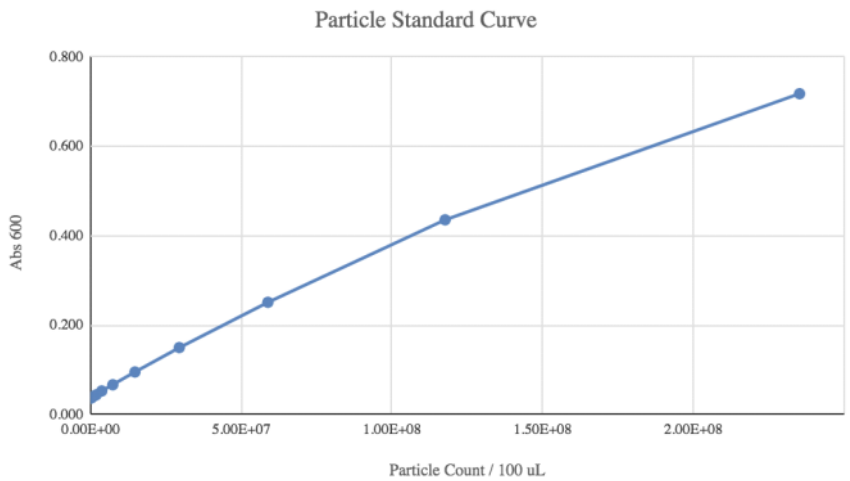
Experimental Data

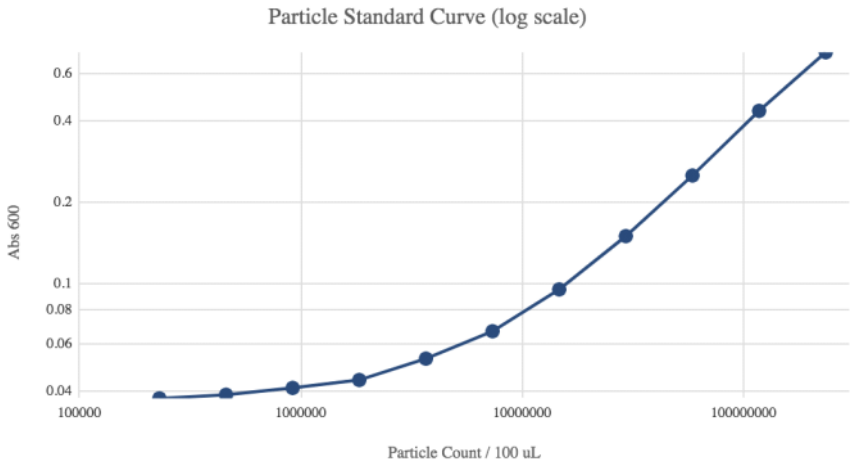
OD600 reference point

	LUDOX CL-X	H2O	
Replicate 1	0.059	0.036	Enter Abs600 absorbance measurements into blue cells
Replicate 2	0.059	0.036	Gold cells are calculated
Replicate 3	0.059	0.036	
Replicate 4	0.058	0.036	
Arith. Mean	0.059	0.036	
Corrected Abs600	0.023		*Corrected value is particle-only contribution*
Reference OD600	0.063		*Reference value is for 100uL of LUDOX CL-X in a well of a standard 96-well flat-bottom black with clear bottom plate*
OD600/Abs600	2.763		*Corrected value = scaling factor * measured value*
Temperature	26.8		
Bandwidth	9 nm		
Wavelength	600 nm		

Particle Standard Curve

Number of Particles	2.35E+08	1.18E+08	5.88E+07	2.94E+07	1.47E+07	7.35E+06	3.68E+06	1.84E+06	9.19E+05	4.60E+05	2.30E+05	0
Replicate 1	0.753899991	0.416200012	0.228300005	0.141900003	0.092399995	0.062700003	0.049899995	0.042899995	0.039900001	0.038199998	0.037000000	0.036499995
Replicate 2	0.710900008	0.439300000	0.249599993	0.155200004	0.088799998	0.063400000	0.050999995	0.041799995	0.040300000	0.038800001	0.037700001	0.036299995
Replicate 3	0.727900028	0.421200007	0.244900003	0.132799997	0.088100001	0.063699997	0.050999995	0.043200001	0.040899995	0.037599995	0.037000000	0.036400001
Replicate 4	0.675599992	0.464100003	0.280400008	0.168400004	0.110600002	0.076099995	0.058600001	0.047600001	0.042899995	0.040100000	0.038300000	0.036499995
Arith. Mean	0.717	0.435	0.251	0.150	0.095	0.066	0.053	0.044	0.041	0.039	0.038	0.036
Arith. Std.Dev.	0.033	0.022	0.022	0.016	0.011	0.006	0.004	0.003	0.001	0.001	0.001	0.000
Arith. Net Mean	0.681	0.399	0.214	0.113	0.059	0.030	0.016	0.007	0.005	0.002	0.001	





Values should form a straight line on both linear and log scale
Slope should be 1:1
Common problems:
** Consistent pipetting error --> log graph is a straight line but not 1:1 slope*
** Oversaturated detector --> low concentrations linear, but high concentrations saturate or fall*

Cospheric Monodisperse Silica Microspheres 0.961um diameter	
Spheres/gram	1.20E+12
grams/mL	1.8
Spheres/0.55 mL	1.19E+12
Resuspend volume n	2.55
Dilution X:	100
Total volume mL:	255
Particles / mL:	4.71E+09
Well volume (mL)	0.1
Initial particles:	4.71E+08

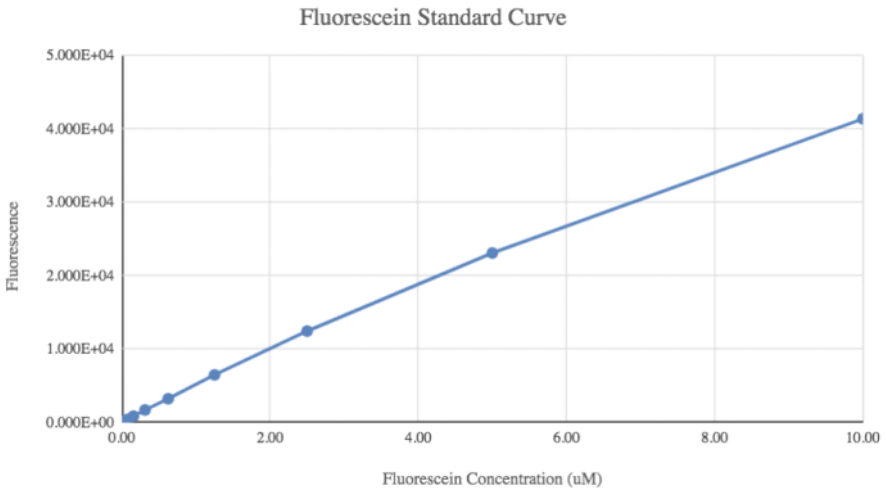
Particles / OD

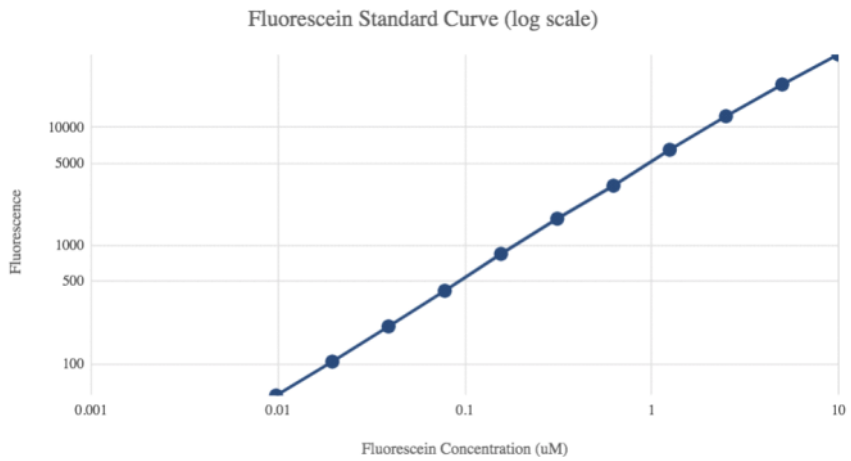
Number of Particles	**2.35E+08**	**1.18E+08**	**5.88E+07**	**2.94E+07**	**1.47E+07**	**7.35E+06**	**3.68E+06**	**1.84E+06**	**9.19E+05**	**4.60E+05**	**2.30E+05**
Mean particles / Abs	3.46E+08	2.95E+08	2.74E+08	2.60E+08	2.51E+08	2.45E+08	2.27E+08	2.47E+08	2.01E+08	2.04E+08	2.14E+08
Mean of med-high levels:		2.65E+08									

Final scaling level determined from medium-high points likely to be less impacted by saturation or pipetting error
If needed, you can shift which points are used, but it is likely better to correct instrument settings and protocol.

Fluorescence Standard Curve

	10.00	**5**	**2.5**	**1.25**	**0.625**	**0.313**	**0.156**	**0.078**	**0.039**	**0.0195**	**0.0098**	**0**
Replicate 1	42359	22744	11886	6418	3207	1568	751	377	176	92	49	1
Replicate 2	39639	23566	12718	6527	3373	1558	799	416	204	104	54	5
Replicate 3	39469	21963	12353	6261	3218	1637	959	416	210	110	56	2
Replicate 4	44091	24093	12830	6713	3087	2016	908	457	243	114	58	4
Arith. Mean	4.139E+04	2.309E+04	1.245E+04	6.480E+03	3.221E+03	1.695E+03	8.543E+02	4.165E+02	2.083E+02	1.050E+02	5.425E+01	3.000E+00
Arith. Std.Dev.	2.235E+03	9.350E+02	4.257E+02	1.900E+02	1.173E+02	2.170E+02	9.587E+01	3.266E+01	2.750E+01	9.592E+00	3.862E+00	1.826E+00
Arith. Net Mean	4.139E+04	2.309E+04	1.244E+04	6.477E+03	3.218E+03	1.692E+03	8.513E+02	4.135E+02	2.053E+02	1.020E+02	5.125E+01	





Values should form a straight line on both linear and log scale
Slope should be 1:1
Common problems:
** Consistent pipetting error --> log graph is a straight line but not 1:1 slope*
** Oversaturated detector --> low concentrations linear, but high concentrations saturate or fall*

Fluorescein uM --> MEFL calculation:	
Initial Molarity	1.00E-05
Molecules / Mole	6.02E+23
Well volume (L):	1.00E-04
Initial Molecules:	6.02E+14
MEFL / uM	6.02E+12

Fluorescein/a.u.											
Fluorescein uM	**10.00**	**5.00**	**2.50**	**1.25**	**0.63**	**0.31**	**0.16**	**0.08**	**0.04**	**0.02**	**0.01**
uM Fluorescein/a.u.	2.42E-04	2.17E-04	2.01E-04	1.93E-04	1.94E-04	1.85E-04	1.84E-04	1.89E-04	1.90E-04	1.91E-04	1.91E-04
Mean uM fluorescein / a.u.:		1.98E-04									
MEFL / a.u.:		1.19E+09									
		Final scaling level determined from medium-high points likely to be less impacted by saturation or pipetting error									
		If needed, you can shift which points are used, but it is likely better to correct instrument settings and protocol.									

Raw Plate Reader Measurements

Raw Plate Readings

If you followed the recommended plate layout:
Copy fluorescence and Abs600 measurements from your plate reader into blue cells
They will automatically propagate into the correct locations in the Fluorescence Measurement Sheet

Fluorescence Raw Readings:

Hour 0:	Neg. Contr	Pos. Contrc	Device 1	Device 2	Device 3	Device 4	Device 5	Device 6	LB + Chlor (blank)
Colony 1, Replicate	58	83	168	110	57	158	256	87	62
Colony 1, Replicate	54	85	162	107	52	108	246	83	60
Colony 1, Replicate	57	80	119	110	62	97	248	82	60
Colony 1, Replicate	47	82	155	110	44	166	238	78	57
Colony 2, Replicate	52	96	152	107	59	141	222	77	55
Colony 2, Replicate	52	94	148	108	56	134	207	70	55
Colony 2, Replicate	55	96	148	110	58	135	215	73	52
Colony 2, Replicate	60	95	141	108	56	131	204	71	53

Hour 6:	Neg. Contr	Pos. Contrc	Device 1	Device 2	Device 3	Device 4	Device 5	Device 6	LB + Chlor (blank)
Colony 1, Replicate	71	735	582	2193	85	1153	469	601	59
Colony 1, Replicate	75	702	547	2241	84	1319	451	537	58
Colony 1, Replicate	67	727	518	2169	80	1281	467	499	58
Colony 1, Replicate	80	717	509	2104	79	1208	470	579	55
Colony 2, Replicate	72	1003	422	1340	86	684	673	563	59
Colony 2, Replicate	73	1010	421	1393	84	661	595	551	55
Colony 2, Replicate	69	995	407	1376	88	693	684	543	59
Colony 2, Replicate	74	985	381	1355	79	663	620	545	58

Abs600 Raw Readings:

Hour 0:	Neg. Control	Pos. Control	Device 1	Device 2	Device 3	Device 4	Device 5	Device 6	LB + Chlor (blank)
Colony 1, Replicate	0.05700000003	0.0601999983	0.0630000	0.0727000	0.0722000	0.0649000	0.0741000	0.0599999	0.0469000
Colony 1, Replicate	0.05550000072	0.0612000003	0.0582999	0.0680999	0.0694999	0.0573000	0.0710000	0.0588000	0.0469999
Colony 1, Replicate	0.05550000072	0.0595000013	0.0544999	0.0685999	0.0702999	0.0553999	0.0706999	0.0564999	0.0460000
Colony 1, Replicate	0.05270000175	0.0595999993	0.0588000	0.0687000	0.0626000	0.0654999	0.0728000	0.0568000	0.0467999
Colony 2, Replicate	0.07069999725	0.0636999979	0.0582000	0.0716999	0.0676999	0.0579999	0.0709000	0.0637999	0.0463000
Colony 2, Replicate	0.07599999756	0.0648000016	0.0575999	0.0728999	0.0685999	0.0575999	0.0714000	0.0614000	0.0471000
Colony 2, Replicate	0.07310000062	0.0643000006	0.0575000	0.0698999	0.0681999	0.0566999	0.0681999	0.0608000	0.0467000
Colony 2, Replicate	0.07609999925	0.0656000003	0.0566999	0.0720999	0.0671999	0.0577999	0.0702999	0.0631000	0.0482000

Hour 6:	Neg. Control	Pos. Control	Device 1	Device 2	Device 3	Device 4	Device 5	Device 6	LB + Chlor (blank)
Colony 1, Replicate	0.3986999989	0.3668000102	0.0960000	0.4792999	0.4424999	0.1931000	0.0970999	0.3806999	0.0456999
Colony 1, Replicate	0.4169999957	0.3652000129	0.0992999	0.5074999	0.4399999	0.2272000	0.0945999	0.3788999	0.0467000
Colony 1, Replicate	0.3998999894	0.3761000037	0.0947000	0.4850000	0.4282999	0.2143000	0.0956000	0.3612000	0.0450999
Colony 1, Replicate	0.4566999972	0.3594999909	0.0943000	0.4905999	0.4341999	0.2190999	0.0982000	0.4013000	0.0463999
Colony 2, Replicate	0.4246999919	0.3889999986	0.0758000	0.4199999	0.4453000	0.1297000	0.1037999	0.3838	0.0447999
Colony 2, Replicate	0.4284999967	0.3905999959	0.0772000	0.4332999	0.4402999	0.1260000	0.0998999	0.3702000	0.0452999
Colony 2, Replicate	0.4180999994	0.3853999972	0.0738999	0.4122000	0.4368000	0.1294	0.1013000	0.3564999	0.0450000
Colony 2, Replicate	0.4208999872	0.3867000043	0.0747999	0.4147000	0.4343999	0.1288000	0.0992999	0.3540000	0.0467999

Assumed plate well pattern:

A1	A2	A3	A4	A5	A6	A7	A8	A9
B1	B2	B3	B4	B5	B6	B7	B8	B9
C1	C2	C3	C4	C5	C6	C7	C8	C9
D1	D2	D3	D4	D5	D6	D7	D8	D9
E1	E2	E3	E4	E5	E6	E7	E8	E9
F1	F2	F3	F4	F5	F6	F7	F8	F9
G1	G2	G3	G4	G5	G6	G7	G8	G9
H1	H2	H3	H4	H5	H6	H7	H8	H9

Fluorescence per OD

Unit Scaling Factors *These are imported from the prior sheets*

OD600 / Abs600	2.76
uM Fluorescein / a.u.	1.98E-04

Enter fluorescence and Abs600 measurements into blue cells on "Raw Plate Reader Measurements"
Gold cells are calculated from values on other sheets

Experimental Values:

uM Fluorescein / OD

Hour 0:	Neg. Contr	Pos. Contr	Device 1	Device 2	Device 3	Device 4	Device 5	Device 6
Colony 1, Replicate 1	-0.028	0.113	0.471	0.133	-0.014	0.382	0.511	0.137
Colony 1, Replicate 2	-0.051	0.126	0.646	0.160	-0.025	0.334	0.555	0.140
Colony 1, Replicate 3	-0.023	0.106	0.497	0.158	0.006	0.282	0.545	0.150
Colony 1, Replicate 4	-0.121	0.140	0.585	0.173	-0.059	0.417	0.499	0.150
Colony 2, Replicate 1	-0.009	0.169	0.584	0.147	0.013	0.526	0.486	0.090
Colony 2, Replicate 2	-0.007	0.158	0.634	0.147	0.003	0.539	0.448	0.075
Colony 2, Replicate 3	0.008	0.179	0.637	0.179	0.020	0.594	0.543	0.107
Colony 2, Replicate 4	0.018	0.173	0.741	0.165	0.011	0.582	0.489	0.087

Hour 6:	Neg. Contr	Pos. Contr	Device 1	Device 2	Device 3	Device 4	Device 5	Device 6
Colony 1, Replicate 1	0.002	0.151	0.745	0.352	0.005	0.532	0.571	0.116
Colony 1, Replicate 2	0.003	0.145	0.666	0.339	0.005	0.500	0.588	0.103
Colony 1, Replicate 3	0.002	0.145	0.664	0.344	0.004	0.518	0.580	0.100
Colony 1, Replicate 4	0.004	0.151	0.679	0.330	0.004	0.478	0.574	0.106
Colony 2, Replicate 1	0.002	0.196	0.839	0.244	0.005	0.527	0.745	0.106
Colony 2, Replicate 2	0.003	0.198	0.822	0.247	0.005	0.538	0.708	0.109
Colony 2, Replicate 3	0.002	0.197	0.862	0.257	0.005	0.538	0.795	0.111
Colony 2, Replicate 4	0.003	0.195	0.826	0.252	0.004	0.528	0.767	0.114

Net Fluorescein a.u.

Neg. Control	Pos. Control	Device 1	Device 2	Device 3	Device 4	Device 5	Device 6
-4.00	21.00	106.00	48.00	-5.00	96.00	194.00	25.00
-6.00	25.00	102.00	47.00	-8.00	48.00	186.00	23.00
-3.00	20.00	59.00	50.00	2.00	37.00	188.00	22.00
-10.00	25.00	98.00	53.00	-13.00	109.00	181.00	21.00
-3.00	41.00	97.00	52.00	4.00	86.00	167.00	22.00
-3.00	39.00	93.00	53.00	1.00	79.00	152.00	15.00
3.00	44.00	96.00	58.00	6.00	83.00	163.00	21.00
7.00	42.00	88.00	55.00	3.00	78.00	151.00	18.00

Neg. Control	Pos. Control	Device 1	Device 2	Device 3	Device 4	Device 5	Device 6
12.00	676.00	523.00	2134.00	26.00	1094.00	410.00	542.00
17.00	644.00	489.00	2183.00	26.00	1261.00	393.00	479.00
9.00	669.00	460.00	2111.00	22.00	1223.00	409.00	441.00
25.00	662.00	454.00	2049.00	24.00	1153.00	415.00	524.00
13.00	944.00	363.00	1281.00	27.00	625.00	614.00	504.00
18.00	955.00	366.00	1338.00	29.00	606.00	540.00	496.00
10.00	936.00	348.00	1317.00	29.00	634.00	625.00	484.00
16.00	927.00	323.00	1297.00	21.00	605.00	562.00	487.00

Net Abs 600

Neg. Control	Pos. Control	Device 1	Device 2	Device 3	Device 4	Device 5	Device 6
0.010	0.013	0.016	0.026	0.025	0.018	0.027	0.013
0.009	0.014	0.011	0.021	0.023	0.010	0.024	0.012
0.010	0.014	0.008	0.023	0.024	0.009	0.025	0.010
0.006	0.013	0.012	0.022	0.016	0.019	0.026	0.010
0.024	0.017	0.012	0.025	0.021	0.012	0.025	0.017
0.029	0.018	0.010	0.026	0.021	0.010	0.024	0.014
0.026	0.018	0.011	0.023	0.021	0.010	0.021	0.014
0.028	0.017	0.008	0.024	0.019	0.010	0.022	0.015

Neg. Control	Pos. Control	Device 1	Device 2	Device 3	Device 4	Device 5	Device 6
0.353	0.321	0.050	0.434	0.397	0.147	0.051	0.335
0.370	0.319	0.053	0.461	0.393	0.181	0.048	0.332
0.355	0.331	0.050	0.440	0.383	0.169	0.051	0.316
0.410	0.313	0.048	0.444	0.388	0.173	0.052	0.355
0.380	0.344	0.031	0.375	0.401	0.085	0.059	0.339
0.383	0.345	0.032	0.388	0.395	0.081	0.055	0.325
0.373	0.340	0.029	0.367	0.392	0.084	0.056	0.311
0.374	0.340	0.028	0.368	0.388	0.082	0.052	0.307

Fluorescence per particle

Unit Scaling Factors *These are imported from the prior sheets*

Particles / Abs600	2.65E+08
MEFL / a.u.	1.19E+09

Enter fluorescence and Abs600 measurements into blue cells on "Raw Plate Reader Measurements"

Gold cells are calculated from values on other sheets

Experimental Values:

MEFL / particle

Hour 0:	Neg. Control	Pos. Control	Device 1	Device 2	Device 3	Device 4	Device 5	Device 6
Colony 1, Replicate 1	-1.78E+03	7.10E+03	2.96E+04	8.36E+03	-8.89E+02	2.40E+04	3.21E+04	8.58E+03
Colony 1, Replicate 2	-3.17E+03	7.92E+03	4.06E+04	1.00E+04	-1.60E+03	2.10E+04	3.48E+04	8.76E+03
Colony 1, Replicate 3	-1.42E+03	6.66E+03	3.12E+04	9.95E+03	3.70E+02	1.77E+04	3.42E+04	9.42E+03
Colony 1, Replicate 4	-7.62E+03	8.78E+03	3.67E+04	1.09E+04	-3.70E+03	2.62E+04	3.13E+04	9.44E+03
Colony 2, Replicate 1	-5.53E+02	1.06E+04	3.66E+04	9.20E+03	8.40E+02	3.30E+04	3.05E+04	5.65E+03
Colony 2, Replicate 2	-4.67E+02	9.91E+03	3.98E+04	9.24E+03	2.09E+02	3.38E+04	2.81E+04	4.72E+03
Colony 2, Replicate 3	5.11E+02	1.12E+04	4.00E+04	1.12E+04	1.25E+03	3.73E+04	3.41E+04	6.70E+03
Colony 2, Replicate 4	1.13E+03	1.09E+04	4.65E+04	1.03E+04	7.10E+02	3.65E+04	3.07E+04	5.43E+03

Hour 6:	Neg. Control	Pos. Control	Device 1	Device 2	Device 3	Device 4	Device 5	Device 6
Colony 1, Replicate 1	1.53E+02	9.47E+03	4.67E+04	2.21E+04	2.95E+02	3.34E+04	3.59E+04	7.27E+03
Colony 1, Replicate 2	2.06E+02	9.09E+03	4.18E+04	2.13E+04	2.97E+02	3.14E+04	3.69E+04	6.48E+03
Colony 1, Replicate 3	1.14E+02	9.09E+03	4.17E+04	2.16E+04	2.58E+02	3.25E+04	3.64E+04	6.27E+03
Colony 1, Replicate 4	2.74E+02	9.51E+03	4.26E+04	2.07E+04	2.78E+02	3.00E+04	3.60E+04	6.64E+03
Colony 2, Replicate 1	1.54E+02	1.23E+04	5.26E+04	1.54E+04	3.03E+02	3.31E+04	4.68E+04	6.68E+03
Colony 2, Replicate 2	2.11E+02	1.24E+04	5.16E+04	1.55E+04	3.30E+02	3.38E+04	4.45E+04	6.86E+03
Colony 2, Replicate 3	1.21E+02	1.24E+04	5.41E+04	1.61E+04	3.33E+02	3.38E+04	4.99E+04	6.99E+03
Colony 2, Replicate 4	1.92E+02	1.23E+04	5.19E+04	1.59E+04	2.44E+02	3.32E+04	4.81E+04	7.13E+03

Net Fluorescein a.u.

Neg. Control	Pos. Control	Device 1	Device 2	Device 3	Device 4	Device 5	Device 6
-4.00	21.00	106.00	48.00	-5.00	96.00	194.00	25.00
-6.00	25.00	102.00	47.00	-8.00	48.00	186.00	23.00
-3.00	20.00	59.00	50.00	2.00	37.00	188.00	22.00
-10.00	25.00	98.00	53.00	-13.00	109.00	181.00	21.00
-3.00	41.00	97.00	52.00	4.00	86.00	167.00	22.00
-3.00	39.00	93.00	53.00	1.00	79.00	152.00	15.00
3.00	44.00	96.00	58.00	6.00	83.00	163.00	21.00
7.00	42.00	88.00	55.00	3.00	78.00	151.00	18.00

Neg. Control	Pos. Control	Device 1	Device 2	Device 3	Device 4	Device 5	Device 6
12.00	676.00	523.00	2134.00	26.00	1094.00	410.00	542.00
17.00	644.00	489.00	2183.00	26.00	1261.00	393.00	479.00
9.00	669.00	460.00	2111.00	22.00	1223.00	409.00	441.00
25.00	662.00	454.00	2049.00	24.00	1153.00	415.00	524.00
13.00	944.00	363.00	1281.00	27.00	625.00	614.00	504.00
18.00	955.00	366.00	1338.00	29.00	606.00	540.00	496.00
10.00	936.00	348.00	1317.00	29.00	634.00	625.00	484.00
16.00	927.00	323.00	1297.00	21.00	605.00	562.00	487.00

Net Abs 600

Neg. Control	Pos. Control	Device 1	Device 2	Device 3	Device 4	Device 5	Device 6
0.010	0.013	0.016	0.026	0.025	0.018	0.027	0.013
0.009	0.014	0.011	0.021	0.023	0.010	0.024	0.012
0.010	0.014	0.008	0.023	0.024	0.009	0.025	0.010
0.006	0.013	0.012	0.022	0.016	0.019	0.026	0.010
0.024	0.017	0.012	0.025	0.021	0.012	0.025	0.017
0.029	0.018	0.010	0.026	0.021	0.010	0.024	0.014
0.026	0.018	0.011	0.023	0.021	0.010	0.021	0.014
0.028	0.017	0.008	0.024	0.019	0.010	0.022	0.015

Neg. Control	Pos. Control	Device 1	Device 2	Device 3	Device 4	Device 5	Device 6
0.353	0.321	0.050	0.434	0.397	0.147	0.051	0.335
0.370	0.319	0.053	0.461	0.393	0.181	0.048	0.332
0.355	0.331	0.050	0.440	0.383	0.169	0.051	0.316
0.410	0.313	0.048	0.444	0.388	0.173	0.052	0.355
0.380	0.344	0.031	0.375	0.401	0.085	0.059	0.339
0.383	0.345	0.032	0.388	0.395	0.081	0.055	0.325
0.373	0.340	0.029	0.367	0.392	0.084	0.056	0.311
0.374	0.340	0.028	0.368	0.388	0.082	0.052	0.307

Reflection

We were able to follow the given procedure and obtain reasonable data.

However, during the Interlab study, we encountered several issues that needed to be addressed so that we could still produce standardized results. We were unable to turn off pathlength correction on our plate reader, so we standardized our measurements by adjusting the gain to be the same for all fluorescence measurements. A constant gain value was set manually to 57 for the raw plate reader measurements. This gain value was based off of the optimal gain value of the fluorescein standard curve.

Another issue was that the bacteria from Device 3 did not appear to fluoresce as much as the other devices, causing us to speculate that there could have been contamination or a misexpressed gene due to mutation. In order to find an answer, we sent the DNA for sequencing. The sequencing result indicated that the transformation was successful and that there were no evident mutation or contamination. Data from last

year’s Interlab also suggested that Device 3’s fluorescence should be lower than most other devices. Overall, we had a lot of fun throughout the experiment, and we hope that our efforts can contribute to standardizing a measurement process for fluorescence that can be compared across different labs.

MATERIALS

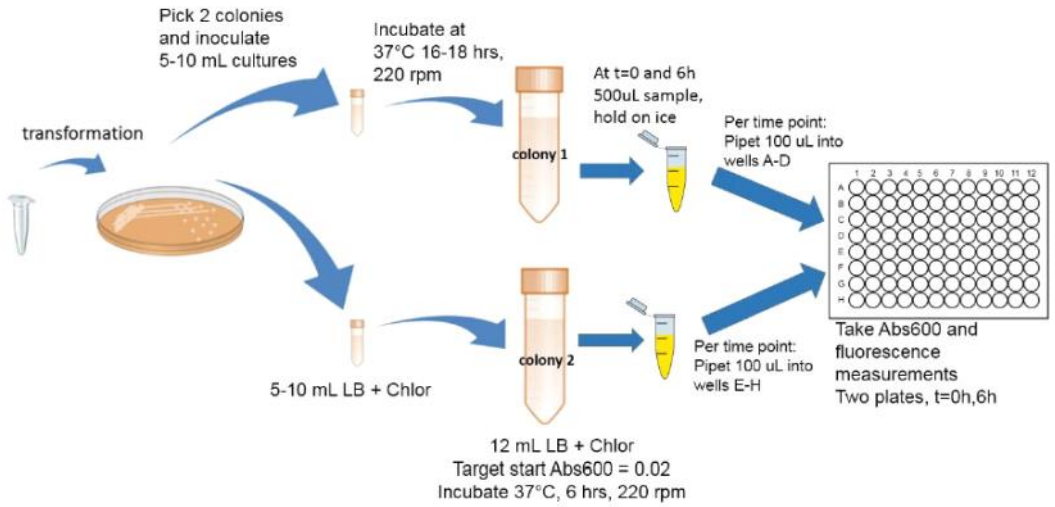
Micro-organism: *Escherichia coli* DH5α strains carrying:

- Positive control: BBa_I20270
- Negative control: BBa_R0040
- Device 1: BBa_J364000
- Device 2: BBa_J364001
- Device 3: BBa_J364002
- Device 4: BBa_J364007
- Device 5: BBa_J364008
- Device 6: BBa_J364009

Plate Reader Settings

- Brand & model: Tecan Infinite 200 PRO
- Absorbance wavelength: 600 nm
- Emission Wavelength: 530 nm
- Excitation Wavelength: 485 nm
- Temperature range: 25-27°C
- Fluorescence reading: Top Optics
- Fluorescence gains: 57
- No filter
- Plate type used: clear

METHODS OVERVIEW



The protocol was provided by iGEM HQ: http://2018.igem.org/wiki/images/0/09/2018_InterLab_Plate_Reader_Protocol.pdf

Problems and Solutions

- Unable to turn off Pathlength correction
  - We could not turn off the pathlength setting on our plate reader, so we standardized our measurements by adjusting the gain to be the same for all fluorescence measurements.
- Consistency of the Gains
  - A constant gain value was set manually to 57 for the raw plate reader measurements. This gain value was based off of the optimal gain value of the fluorescein standard curve.
- Device 3 -> sequencing

        - Bacteria from device 3 did not appear to fluoresce under the naked eye, therefore our team speculated that there could have been contamination or a misexpressed gene due to mutation. In order to find an answer, we purified DNA from Bacteria via miniprep, then sent the purified DNA to a sequencing company. The sequencing result indicated that the transformation was successful and that there were no evident mutation or contamination. Data from last year's interlab also suggested that Device 3's fluorescence should be lower than most other devices.
    - 1 of the liquid cultures did not grow as much
        - We ran the experiment twice, and all of the liquid cultures were turbid in our 2nd trial.
    - CFU did not decrease 10 fold when subjected to a 1:10 dilution

Conclusion

- Device 1: Did not experience much growth; however, Fluorescence and Fluorescence/OD increased drastically.
- Device 2: Above average growth. Highest fluorescence out of all devices after 6 hours. Fluorescence/OD increased.
- Device 3: Normal growth. Poor fluorescence.
- Device 4: Below average growth. Significant increase of fluorescence from 0 to 6 hours. Fluorescence/OD didn't change much.
- Device 5: Not much growth. Fluorescence and Fluorescence/OD increased from 0 to 6 hours.
- Device 6: Normal growth. Fluorescence increased from 0 to 6 hours, but Fluorescence/OD didn't change much from 0 to 6 hours.
- CFU is not a reliable way of determining the number of bacteria cells in a liquid medium as it is subjected to discrepancies
- The reliability of a relative cell count modeled by silica beads cannot be determined as data from other labs were not examined

It is with high expectations and enthusiasm that we took part in the Fifth International InterLab Measurement Study, which aims to provide understanding of a standardized measurement process that can be replicated under the same conditions. To accomplish this goal, all iGEM teams had to measure both the fluorescence and absorbance of 6 Devices using a plate reader.

Methods

"We followed the protocol provided by iGEM HQ."

Equipment and Material Information

Materials

Micro-organism: *Escherichia coli* DH5α strains

Positive control: BBa_I20270

http://parts.igem.org/Part:BBa_I20270

Negative control: BBa_R0040

http://parts.igem.org/Part:BBa_R0040

Device 1: BBa_J364000

http://parts.igem.org/Part:BBa_J364000

Device 2: BBa_J364001

http://parts.igem.org/Part:BBa_J364001

Device 3: BBa_J364002

http://parts.igem.org/Part:BBa_J364002

Device 4: BBa_J364007

http://parts.igem.org/Part:BBa_J364007

Device 5: BBa_J364008

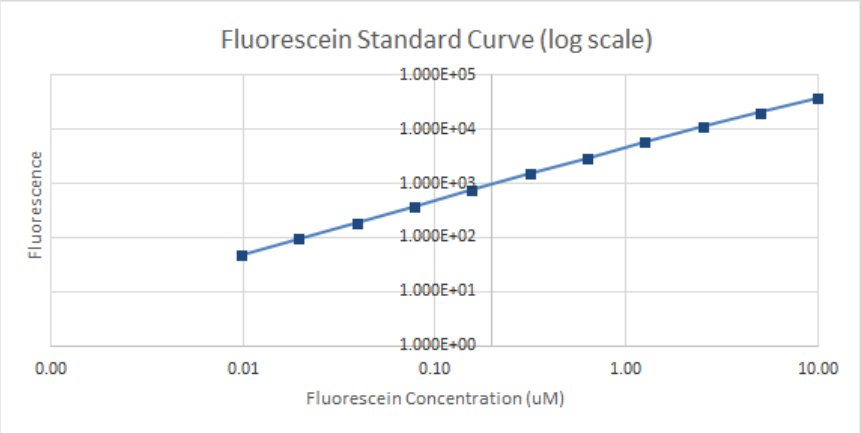
http://parts.igem.org/Part:BBa_J364008

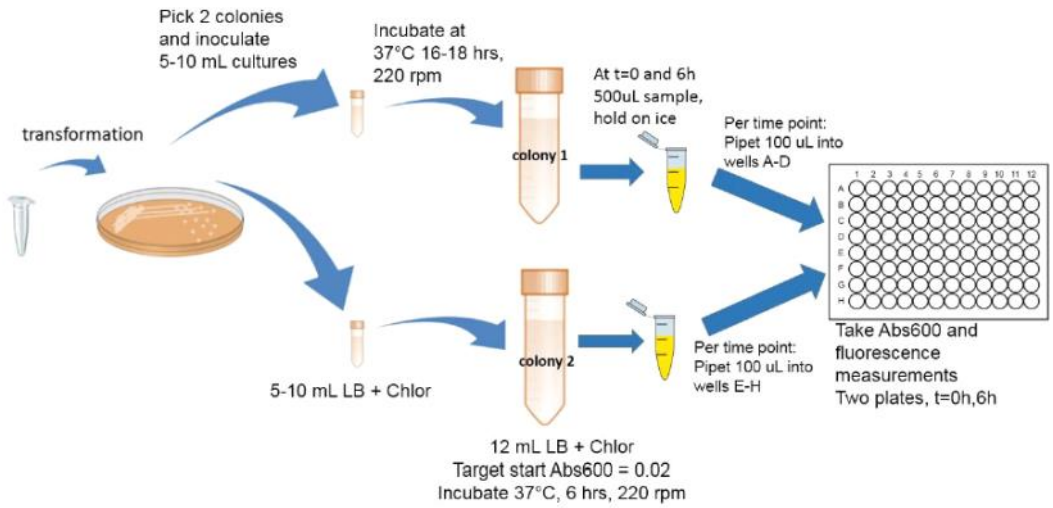
Device 6: BBa_J364009

http://parts.igem.org/Part:BBa_J364009

Plate reader settings

Device 6: BBa_J364009
http://parts.igem.org/Part:BBa_J364009
Plate reader settings
Settings
Brand & model: Tecan Infinite 200 PRO
Absorbance wavelength: 600 nm
Emission Wavelength: 530 nm
Excitation Wavelength: 485 nm
Temperature range: 25 - 27 C
Fluorescence reading: Top Optics
Fluorescence gains: 57
No filter
Plate type: Clear
Data
Key data points (graphs of standard curve)
Fluorescein Standard Curve (log scale)





- Goal

iGEM 2018 InterLab Study Protocol

Before You Begin

Read through this entire protocol sheet carefully before you start your experiment and prepare any materials you may need. In order to improve reproducibility, **we are requiring all participating teams to use plate readers to take measurements of fluorescence and absorbance**. If you do not have access to a plate reader with those capabilities, you may collaborate with another team. If the plate reader requirement is a significant barrier for your team, you can still participate in the InterLab study. Contact the iGEM Measurement Committee at measurement at igem dot org to discuss your situation.

Before beginning your experiments, it will be helpful to gather the following information about your plate reader, as you will be asked to provide this information when submitting your data to iGEM HQ:

- ❑ Instrument brand and model _____
- ❑ Can your instrument measure both absorbance and fluorescence? _____
- ❑ Does your instrument have pathlength correction, and if yes can it be disabled? _____
- ❑ Does your instrument have variable temperature settings, and if yes can this be set to room temperature? _____
- ❑ What filters does your instrument have for measuring GFP? You will need information about the bandpass width (530 nm / 30 nm bandpass, 25-30nm width is recommended), excitation (485 nm is recommended) and emission (520-530 nm is recommended) of this filter. _____
- ❑ Does your instrument use top or bottom optics (i.e. does your plate reader read samples from the top of the plate or the bottom)? _____

You will need all of the following supplies and reagents to complete this entire protocol. Please take a moment to check that you have all of these supplies and reagents before you begin:

- ❑ Measurement Kit (provided with the iGEM distribution shipment) containing:
  - ❑ 1ml LUDOX CL-X
  - ❑ 150 µL Silica Bead (microsphere suspension)
  - ❑ Fluorescein (powder, in amber tube)

- ❑ iGEM Parts Distribution Kit Plates (you will obtain the test devices from the parts kit plates)
- ❑ 1x PBS (phosphate buffered saline, pH 7.4 - 7.6)
- ❑ ddH_2O (ultrapure filtered or double distilled water)
- ❑ Competent cells (*Escherichia coli* strain DH5 α)
- ❑ LB (Luria Bertani) media
- ❑ Chloramphenicol (stock concentration 25 mg/mL dissolved in EtOH)
- ❑ 50 ml Falcon tube (or equivalent, preferably amber or covered in foil to block light)
- ❑ Incubator at 37°C
- ❑ 1.5 ml eppendorf tubes
- ❑ Ice bucket with ice
- ❑ Micropipettes (capable of pipetting a range of volumes between 1 µL and 1000 µL)
- ❑ Micropipette tips
- ❑ 96 well plates, black with clear flat bottom preferred, at least 3-4 plates (provided by team)

Calibration Protocols

CALIBRATION PROTOCOLS SHOULD BE COMPLETED BEFORE CELL MEASUREMENTS ARE TAKEN!

You will make three sets of unit calibration measurements: an OD_{600} reference point, a particle standard curve, and a fluorescein standard curve. Before beginning these protocols, please ensure that you are familiar with the measurement modes and settings of your instrument.

For all of these calibration measurements, you must use the same plates and volumes that you will use in your cell-based assays. You must also use the same settings (e.g., filters or excitation and emission wavelengths) that you will use in your cell-based assays. **If you do not use the same plates, volumes, and settings, the calibration will not be valid.** Make sure to record all information about your instrument (checklist on page 1 of this protocol) as these will be required later when you document your experiment. If your instrument has variable temperature settings, the instrument temperature should be set to room temperature (approximately 20-25 C) for all measurements.

Calibration 1: OD_{600} Reference point - LUDOX Protocol

You will use LUDOX CL-X (45% colloidal silica suspension) as a single point reference to obtain a conversion factor to transform your absorbance (Abs_{600}) data from your plate reader into a comparable OD_{600} measurement as would be obtained in a spectrophotometer. Such conversion is

necessary because plate reader measurements of absorbance are volume dependent; the depth of the fluid in the well defines the path length of the light passing through the sample, which can vary slightly from well to well. In a standard spectrophotometer, the path length is fixed and is defined by the width of the cuvette, which is constant. Therefore this conversion calculation can transform Abs_{600} measurements from a plate reader (i.e., absorbance at 600nm, the basic output of most instruments) into comparable OD_{600} measurements. The LUDOX solution is only weakly scattering and so will give a low absorbance value.

[**IMPORTANT NOTE**: many plate readers have an automatic path length correction feature. This adjustment compromises the accuracy of measurement in highly light scattering solutions, such as dense cultures of cells. **YOU MUST THEREFORE TURN OFF PATHLENGTH CORRECTION if it can be disabled on your instrument**.]

Materials:

1ml LUDOX CL-X (provided in kit)
ddH_20 (provided by team)
96 well plate, black with clear flat bottom preferred (provided by team)

Method

- ❑ Add 100 µl LUDOX into wells A1, B1, C1, D1
- ❑ Add 100 µl of dd H_2O into wells A2, B2, C2, D2
- ❑ Measure absorbance at 600 nm of all samples in the measurement mode you plan to use for cell measurements
- ❑ Record the data in the table below or in your notebook
- ❑ Import data into Excel sheet provided (**OD600 reference point tab**)

	LUDOX CL-X	ddH_2O
replicate 1		
replicate 2		
replicate 3		
replicate 4		

	A	B	C	D
1		LUDOX CL-X	H2O	
2	Replicate 1	0.078	0.038	
3	Replicate 2	0.077	0.038	
4	Replicate 3	0.078	0.038	
5	Replicate 4	0.078	0.038	
6	Arith. Mean	0.078	0.038	
7	Corrected Abs600	0.040		
8	Reference OD600	0.063		
9	OD600/Abs600	1.585		
10				

The screen capture image above is from the OD600 Reference Point tab of the InterLab Excel sheet. The table shows the data for OD_{600} measured by a spectrophotometer (row 8, yellow box, "Reference OD600") and plate reader data for the H_2O and LUDOX similar to what you will likely collect (you will place your own data in the blue boxes). The corrected Abs_{600} is calculated by subtracting the H_2O reading. The reference OD_{600} is defined as that measured by the reference spectrophotometer (as provided to you in the Excel sheet). The correction factor to convert measured Abs_{600} to OD_{600} is thus the Reference OD_{600} divided by Abs_{600}. ***All cell density readings using this instrument with the same settings and volume can be converted to OD_{600} by multiplying by (in this example) 1.585.***

Calibration 2: Particle Standard Curve - Microsphere Protocol

You will prepare a dilution series of monodisperse silica microspheres and measure the Abs_{600} in your plate reader. The size and optical characteristics of these microspheres are similar to cells, and there is a known amount of particles per volume. This measurement will allow you to construct a standard curve of particle concentration which can be used to convert Abs_{600} measurements to an estimated number of cells.

Materials:
300 µL Silica beads - Microsphere suspension (provided in kit, 4.7 x 10^8 microspheres)
ddH_2O (provided by team)
96 well plate, black with clear flat bottom preferred (provided by team)

Method:

Prepare the Microsphere Stock Solution:

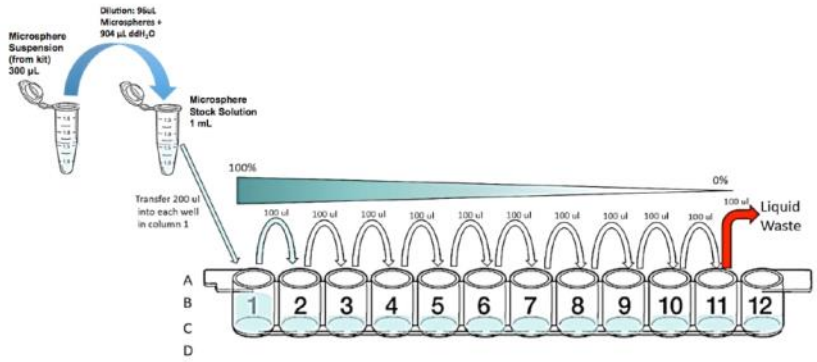
- ❑ Obtain the tube labeled "Silica Beads" from the InterLab test kit and vortex

vigorously for 30 seconds. **NOTE: Microspheres should NOT be stored at 0°C or below**, as freezing affects the properties of the microspheres. If you believe your microspheres may have been frozen, please contact the iGEM Measurement Committee for a replacement (measurement at igem dot org).

- ❑ Immediately pipet 96 µL microspheres into a 1.5 mL eppendorf tube
- ❑ Add 904 µL of ddH_2O to the microspheres
- ❑ Vortex well. This is your Microsphere Stock Solution.

Prepare the serial dilution of Microspheres:

Accurate pipetting is essential. Serial dilutions will be performed across columns 1-11. COLUMN 12 MUST CONTAIN ddH_2O ONLY. Initially you will setup the plate with the microsphere stock solution in column 1 and an equal volume of 1x ddH_2O in columns 2 to 12. You will perform a serial dilution by consecutively transferring 100 µl from column to column with good mixing.



- ❑ **Add 100 µl of ddH_2O** into wells A2, B2, C2, D2....A12, B12, C12, D12
- ❑ **Vortex the tube containing the stock solution of microspheres vigorously for 10 seconds**
- ❑ **Immediately add 200 µl of microspheres stock solution into A1**
- ❑ Transfer 100 µl of microsphere stock solution from A1 into A2.
- ❑ Mix A2 by pipetting up and down 3x and transfer 100 µl into A3...
- ❑ Mix A3 by pipetting up and down 3x and transfer 100 µl into A4...
- ❑ Mix A4 by pipetting up and down 3x and transfer 100 µl into A5...
- ❑ Mix A5 by pipetting up and down 3x and transfer 100 µl into A6...
- ❑ Mix A6 by pipetting up and down 3x and transfer 100 µl into A7...
- ❑ Mix A7 by pipetting up and down 3x and transfer 100 µl into A8...

- ❑ Mix A8 by pipetting up and down 3x and transfer 100 µl into A9...
- ❑ Mix A9 by pipetting up and down 3x and transfer 100 µl into A10...
- ❑ Mix A10 by pipetting up and down 3x and transfer 100 µl into A11...
- ❑ Mix A11 by pipetting up and down 3x and transfer 100 µl into **liquid waste**

TAKE CARE NOT TO CONTINUE SERIAL DILUTION INTO COLUMN 12.

- ❑ Repeat dilution series for rows B, C, D
- ❑ **IMPORTANT!** Re-Mix (Pipette up and down) each row of your plate ***immediately before*** putting in the plate reader! (This is important because the beads begin to settle to the bottom of the wells within about 10 minutes, which will affect the measurements.) Take care to mix gently and avoid creating bubbles on the surface of the liquid.
- ❑ Measure Abs_{600} of all samples in instrument
- ❑ Record the data in your notebook
- ❑ Import data into Excel sheet provided (**particle standard curve tab**)

Calibration 3: Fluorescence standard curve - Fluorescein Protocol

Plate readers report fluorescence values in arbitrary units that vary widely from instrument to instrument. Therefore absolute fluorescence values cannot be directly compared from one instrument to another. In order to compare fluorescence output of test devices between teams, it is necessary for each team to create a standard fluorescence curve. Although distribution of a known concentration of GFP protein would be an ideal way to standardize the amount of GFP fluorescence in our *E. coli* cells, the stability of the protein and the high cost of its purification are problematic. We therefore use the small molecule fluorescein, which has similar excitation and emission properties to GFP, but is cost-effective and easy to prepare. (The version of GFP used in the devices, GFP mut3b, has an excitation maximum at 501 nm and an emission maximum at 511 nm; fluorescein has an excitation maximum at 494 nm and an emission maximum at 525nm).

You will prepare a dilution series of fluorescein in four replicates and measure the fluorescence in a 96 well plate in your plate reader. By measuring these in your plate reader, you will generate a standard curve of fluorescence for fluorescein concentration. You will be able to use this to convert your cell based readings to an equivalent fluorescein concentration. Before beginning this protocol, ensure that you are familiar with the GFP settings and measurement modes of your instrument. You will need to know what filters your instrument has for measuring GFP, including information about the bandpass width (530 nm / 30 nm bandpass, 25-30nm width is recommended), excitation (485 nm is recommended) and emission (520-530 nm is recommended) of this filter.

Materials:

Fluorescein (provided in kit)
10ml 1xPBS pH 7.4-7.6 (phosphate buffered saline; provided by team)

96 well plate, black with clear flat bottom (provided by team)

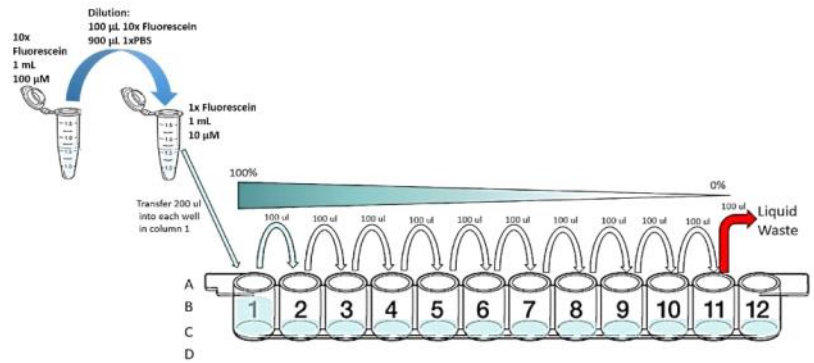
Method

Prepare the fluorescein stock solution:

- ❑ Spin down fluorescein kit tube to make sure pellet is at the bottom of tube.
- ❑ Prepare 10x fluorescein stock solution (100 µM) by resuspending fluorescein in 1 mL of 1xPBS. [**Note**: it is important that the fluorescein is properly dissolved. To check this, after the resuspension you should pipette up and down and examine the solution in the pipette tip – if any particulates are visible in the pipette tip continue to mix the solution until they disappear.]
- ❑ Dilute the 10x fluorescein stock solution with 1xPBS to make a 1x fluorescein solution with concentration 10 µM: 100 µL of 10x fluorescein stock into 900 µL 1x PBS

Prepare the serial dilutions of fluorescein:

Accurate pipetting is essential. Serial dilutions will be performed across columns 1-11. COLUMN 12 MUST CONTAIN PBS BUFFER ONLY. Initially you will setup the plate with the fluorescein stock in column 1 and an equal volume of 1xPBS in columns 2 to 12. You will perform a serial dilution by consecutively transferring 100 µl from column to column with good mixing.



- ❑ **Add 100 µl of PBS** into wells A2, B2, C2, D2....A12, B12, C12, D12
- ❑ **Add 200 µl of fluorescein 1x stock** solution into A1, B1, C1, D1
- ❑ Transfer 100 µl of fluorescein stock solution from A1 into A2.
- ❑ Mix A2 by pipetting up and down 3x and transfer 100 µl into A3...
- ❑ Mix A3 by pipetting up and down 3x and transfer 100 µl into A4...

- ❑ Mix A4 by pipetting up and down 3x and transfer 100 µl into A5...
- ❑ Mix A5 by pipetting up and down 3x and transfer 100 µl into A6...
- ❑ Mix A6 by pipetting up and down 3x and transfer 100 µl into A7...
- ❑ Mix A7 by pipetting up and down 3x and transfer 100 µl into A8...
- ❑ Mix A8 by pipetting up and down 3x and transfer 100 µl into A9...
- ❑ Mix A9 by pipetting up and down 3x and transfer 100 µl into A10...
- ❑ Mix A10 by pipetting up and down 3x and transfer 100 µl into A11...
- ❑ Mix A11 by pipetting up and down 3x and transfer 100 µl into **liquid waste**

TAKE CARE NOT TO CONTINUE SERIAL DILUTION INTO COLUMN 12.

- ❑ Repeat dilution series for rows B, C, D
- ❑ Measure fluorescence of all samples in instrument
- ❑ Record the data in your notebook
- ❑ Import data into Excel sheet provided (**fluorescein standard curve tab**)

Cell measurement protocol

Prior to performing the cell measurements you should perform all three of the calibration measurements. Please do not proceed unless you have completed the three calibration protocols.

Completion of the calibrations will ensure that you understand the measurement process and that you can take the cell measurements under the same conditions. For the sake of consistency and reproducibility, we are requiring all teams to use *E. coli* K-12 DH5-alpha. If you do not have access to this strain, you can request streaks of the transformed devices from another team near you, and this can count as a collaboration as long as it is appropriately documented on both teams' wikis. If you are absolutely unable to obtain the DH5-alpha strain, you may still participate in the InterLab study by contacting the Measurement Committee (measurement at igem dot org) to discuss your situation.

For all of these cell measurements, you must use the same plates and volumes that you used in your calibration protocol. You must also use the same settings (e.g., filters or excitation and emission wavelengths) that you used in your calibration measurements. If you do not use the same plates, volumes, and settings, the measurements will not be valid.

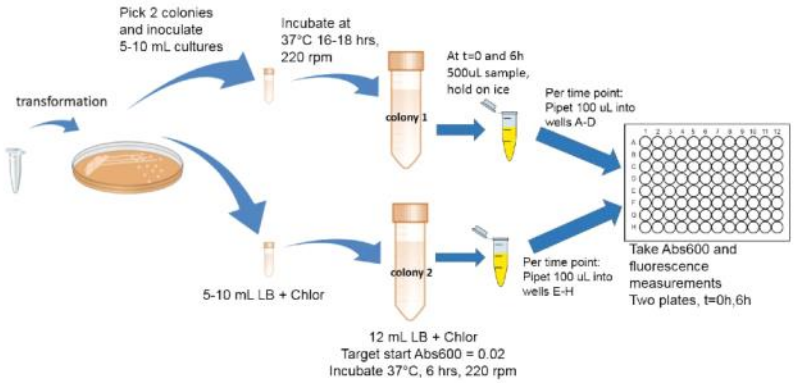
Materials:

Competent cells (*Escherichia coli* strain DH5 α)
LB (Luria Bertani) media
Chloramphenicol (stock concentration 25 mg/mL dissolved in EtOH)
50 ml Falcon tube (or equivalent, preferably amber or covered in foil to block light)
Incubator at 37°C
1.5 ml eppendorf tubes for sample storage
Ice bucket with ice
Micropipettes and tips
96 well plate, black with clear flat bottom preferred (provided by team)

Devices (from Distribution Kit, all in pSB1C3 backbone):

Device	Part Number	Plate	Location
Negative control	BBa_R0040	Kit Plate 7	Well 2D
Positive control	BBa_I20270	Kit Plate 7	Well 2B
Test Device 1	BBa_J364000	Kit Plate 7	Well 2F
Test Device 2	BBa_J364001	Kit Plate 7	Well 2H
Test Device 3	BBa_J364002	Kit Plate 7	Well 2J
Test Device 4	BBa_J364007	Kit Plate 7	Well 2L
Test Device 5	BBa_J364008	Kit Plate 7	Well 2N
Test Device 6	BBa_J364009	Kit Plate 7	Well 2P

Workflow



Method

Day 1: transform *Escherichia coli* DH5 α with these following plasmids (all in pSB1C3):

Device	Part Number	Plate	Location
Negative control	BBa_R0040	Kit Plate 7	Well 2D
Positive control	BBa_I20270	Kit Plate 7	Well 2B
Test Device 1	BBa_J364000	Kit Plate 7	Well 2F
Test Device 2	BBa_J364001	Kit Plate 7	Well 2H
Test Device 3	BBa_J364002	Kit Plate 7	Well 2J
Test Device 4	BBa_J364007	Kit Plate 7	Well 2L
Test Device 5	BBa_J364008	Kit Plate 7	Well 2N
Test Device 6	BBa_J364009	Kit Plate 7	Well 2P

Help Debugging Your Transformations:

- We STRONGLY recommend that you use the iGEM protocol to create your competent cells: http://parts.igem.org/Help:Protocols/Competent_Cells
- Once you have created your competent cells, we STRONGLY recommend that you measure the competency of your cells using the Competent Cell Test Kit: http://parts.igem.org/Help:2017_Competent_Cell_Test_Kit
- Finally, we STRONGLY recommend that you closely follow the iGEM protocols for resuspending DNA from the kit plates and performing the transformation: http://parts.igem.org/Help:Protocols/Transformation

Year after year, we have found that most teams are highly successful when they follow these protocols, even if alternative protocols are used within your lab. If you are having trouble transforming your test devices, please try the protocols above.

Day 2: Pick 2 colonies from each of the transformation plates and inoculate in 5-10 mL LB medium + Chloramphenicol. Grow the cells overnight (16-18 hours) at 37°C and 220 rpm.

Day 3: Cell growth, sampling, and assay

- ☐ Make a 1:10 dilution of each overnight culture in LB+Chloramphenicol (0.5mL of culture into 4.5mL of LB+Chlor)
- ☐ Measure Abs_{600} of these 1:10 diluted cultures
- ☐ Record the data in your notebook
- ☐ Dilute the cultures further to a target Abs_{600} of 0.02 in a final volume of **12 ml** LB medium + Chloramphenicol in 50 mL falcon tube (amber, or covered with foil to block light).
- ☐ Take 500 µL samples of the diluted cultures at 0 hours into 1.5 ml eppendorf tubes, prior to incubation. (At each time point 0 hours and 6 hours, you will take a sample from each of the 8 devices, two colonies per device, for a total of 16 eppendorf tubes with 500 µL samples per time point, 32 samples total). Place the samples on ice.
- ☐ Incubate the remainder of the cultures at 37°C and 220 rpm for 6 hours.
- ☐ Take 500 µL samples of the cultures at 6 hours of incubation into 1.5 ml eppendorf tubes. Place samples on ice.
- ☐ At the end of sampling point you need to measure your samples (Abs_{600} and fluorescence measurement), see the below for details.
- ☐ Record data in your notebook
- ☐ Import data into Excel sheet provided (**fluorescence measurement tab**)

Measurement

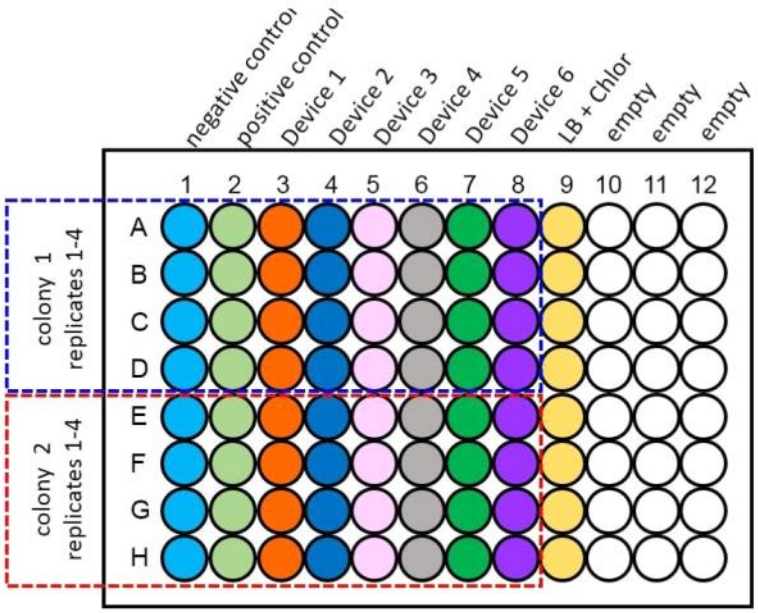
Samples should be laid out according to the plate diagram below. Pipette 100 µl of each sample into each well. From 500 µl samples in a 1.5 ml eppendorf tube, 4 replicate samples of colony #1 should be pipetted into wells in rows A, B, C and D. Replicate samples of colony #2 should be pipetted into wells in rows E, F, G and H. Be sure to include 8 control wells containing 100uL each of only LB+chloramphenicol on each plate in column 9, as shown in the diagram below. Set the instrument settings as those that gave the best results in your calibration curves (no measurements off scale). If necessary you can test more than one of the previously calibrated settings to get the best data (no measurements off scale). Instrument temperature should be set to room temperature (approximately 20-25 C) if your instrument has variable temperature settings.

Help Debugging:

- If you have measurements that are off scale ("OVERFLOW"), that data will not be usable. You need to adjust your settings so that the data will be in range and re-run your calibration.
- If your Abs600 measurements for your cell colonies are very close to that of your LB+Chlor, then your cells have probably not been transformed correctly or grown correctly.
- If your negative and positive control values are very close to each other, that probably means something has gone wrong in your protocol or measurement.

Layout for Abs_{600} and Fluorescence measurement

At the end of the experiment, you should have two plates to read. Each plate should be set up as shown below. You will have one plate for each time point: 0 and 6 hours. On each plate you will read both fluorescence and absorbance.



Protocol: Colony Forming Units per 0.1 OD_{600} *E. coli* cultures

This procedure can be used to calibrate OD_{600} to colony forming unit (CFU) counts, which are directly relatable to the cell concentration of the culture, i.e. viable cell counts per mL. This protocol assumes that 1 bacterial cell will give rise to 1 colony.

For the CFU protocol, you will need to count colonies for your two Positive Control (BBa_I20270) cultures and your two Negative Control (BBa_R0040) cultures.

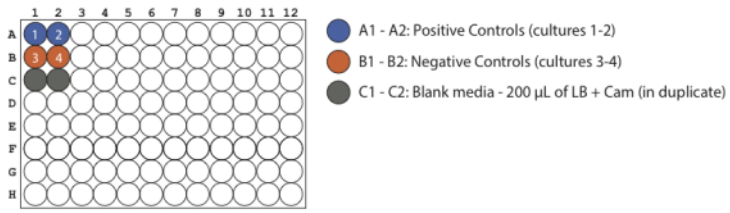
Step 1: Starting Sample Preparation
This protocol will result in CFU/mL for 0.1 OD_{600}. Your overnight cultures will have a much higher OD_{600} and so this section of the protocol, called "Starting Sample Preparation", will give you the "Starting Sample" with a 0.1 OD_{600} measurement.

1. Measure the OD_{600} of your cell cultures, making sure to dilute to the linear detection range of your plate reader, e.g. to 0.05 – 0.5 OD_{600} range. Include blank media (LB + Cam) as well.

   For an overnight culture (16-18 hours of growth), we recommend diluting your culture 1:8 (8-fold dilution) in LB + Cam before measuring the OD_{600}.

   Preparation: Add 25 µL culture to 175 µL LB + Cam in a well in a black 96-well plate, with a clear, flat bottom.

   Recommended plate setup is below. Each well should have 200 µL .



2. Dilute your overnight culture to OD_{600} = 0.1 in 1mL of LB + Cam media. Do this in triplicate for each culture.

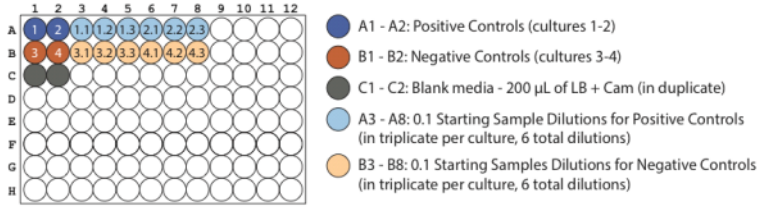
   Use $(C_1)(V_1) = (C_2)(V_2)$ to calculate your dilutions

   C_1 is your starting OD_{600} — C_2 is your target OD_{600} of 0.1
   V_1 is the unknown volume in µL — V_2 is the final volume of 1000 µL

   Important: When calculating C_1, subtract the blank from your reading and multiple by the dilution factor you used.
   Example: C1 = (1:8 OD_{600} - blank OD_{600}) x 8 = (0.195 - 0.042) x 8 = 0.153 x 8 = 1.224

   Example: $(C_1)(V_1) = (C_2)(V_2)$
   $(1.224)(x) = (0.1)(1000\mu L)$
   $x = 100/1.224 = 82$ µL culture
   Add 82 µL of culture to 918 µL media for a total volume of 1000 µL

3. Check the OD_{600} and make sure it is 0.1 (minus the blank measurement).
   Recommended plate setup is below. Each well should have 200 µL .

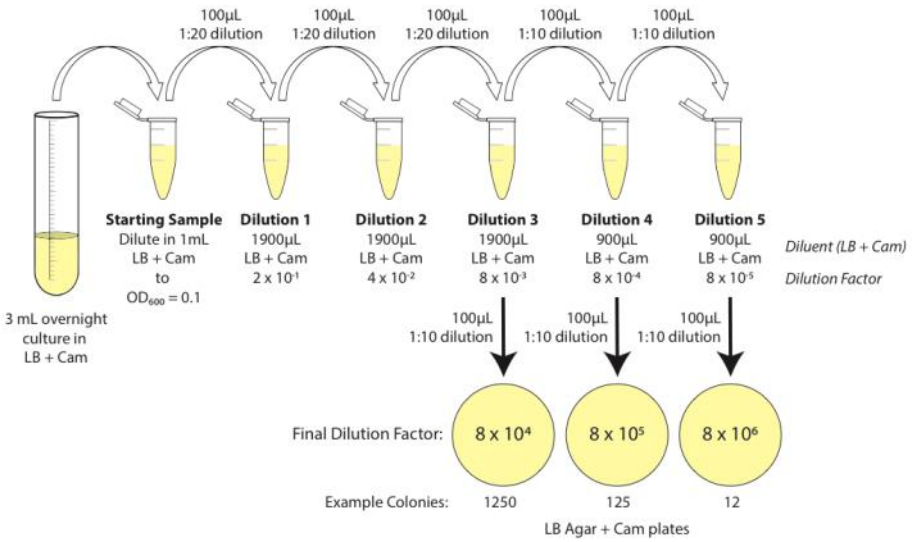


Step 2: Dilution Series Instructions

Do the following serial dilutions for your triplicate Starting Samples you prepared in Step 1. You should have 12 total Starting Samples - 6 for your Positive Controls and 6 for your Negative Controls.

For each Starting Sample (*total for all 12 showed in italics in paraenthesis*):

1. You will need 3 LB Agar + Cam plates (*36 total*).
2. Prepare three 2.0 mL tubes (*36 total*) with 1900 µL of LB + Cam media for Dilutions 1, 2, and 3 (see figure below).
3. Prepare two 1.5 mL tubes (*24 total*) with 900 µL of LB + Cam media for Dilutions 4 and 5 (see figure below)..
4. Label each tube according to the figure below (Dilution 1, etc.) for each Starting Sample.
5. Pipet 100 µL of Starting Culture into Dilution 1. Discard tip. Do NOT pipette up and down. Vortex tube for 5-10 secs.
6. Repeat Step 5 for each dilution through to Dilution 5 as shown below.
7. Aseptically spead plate 100 µL on LB + Cam plates for Dilutions 3, 4, and 5.
8. Incubate at 37°C overnight and count colonies after 18-20 hours of growth.



Step 3: CFU/mL/OD Calculation Instructions

Based on the assumption that 1 bacterial cell gives rise to 1 colony, colony forming units (CFU) per 1mL of an $OD_{600} = 0.1$ culture can be calculated as follows:

1. Count the colonies on each plate with fewer than 300 colonies.
2. Multiple the colony count by the Final Dilution Factor on each plate.

Example using Dilution 4 from above

# colonies	x	Final Dilution Factor	= CFU/mL
125	x	(8×10^5)	= 1×10^8 CFU/mL in Starting Sample ($OD_{600} = 0.1$)