

## INTERLAB

### OD600 Reference point

#### Plate reader

SpectraMax M2 Microplate Readers

Filter: 530nm

#### Materials

1 ml LUDOX

H<sub>2</sub>O

96 well cell culture plate (cleared flat bottom)

#### Method

1. Add 100µl LODOX in to A1, B1, C1, D1 (or 1 mL LUDOX into cuvette)
2. Add 100 µl of H<sub>2</sub>O into wells A2, B2, C2, D2 (or 1 mL H<sub>2</sub>O into cuvette)
3. Measure absorbance 600 nm of all samples in all standard measurement modes in instrument
4. Record the data in excel and Import data into Excel ( OD600 reference point tab )  
Sheet\_1 provided

#### Data Result

	LUDOX-HS4	H2O
Replicate 1	0.056	0.043
Replicate 2	0.055	0.044
Replicate 3	0.052	0.044
Replicate 4	0.052	0.044
Arith. Mean	0.05375	0.04375
Corrected Abs600	0.01	
Reference OD600	0.0425	
OD600/Abs600	4.25	

### Fluorescein fluorescence standard curve

#### Plate reader

SpectraMax M2 Microplate Readers

Emission filter: 530 nm

Excitation filter: 485 nm

#### Materials

Fluorescein

10 ml 1x PBS

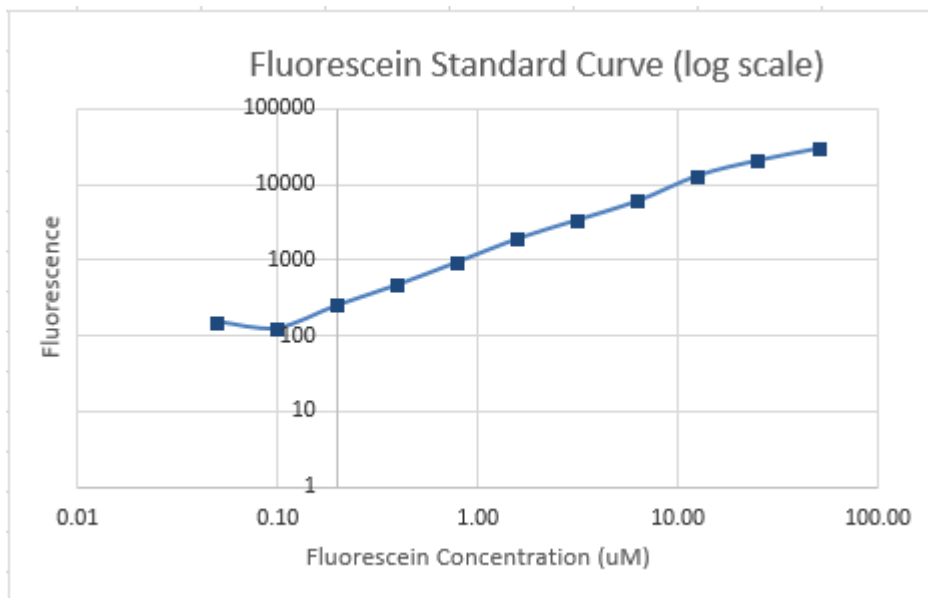
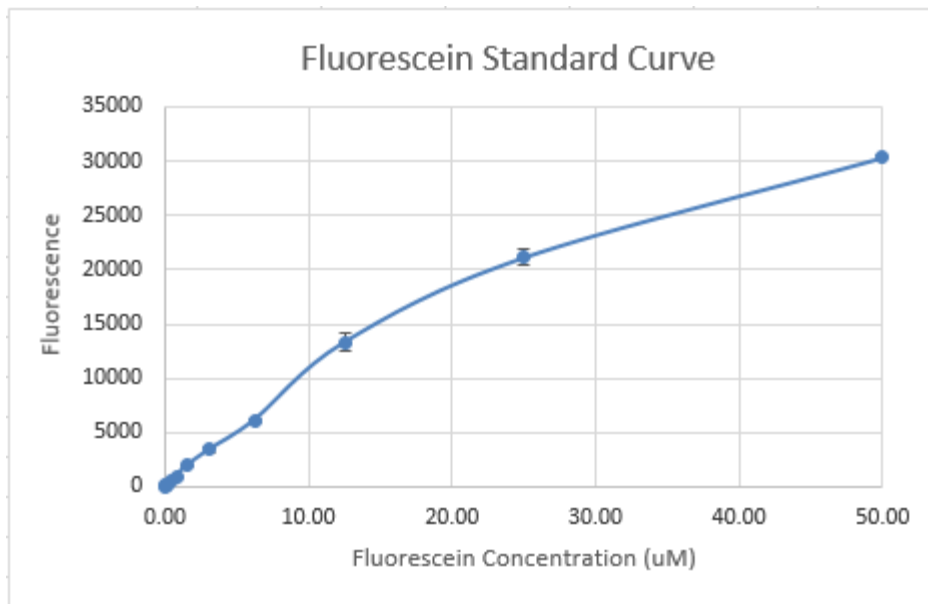
96 well plate (cleared flat bottom)

## Method

- Prepare the fluorescein stock solution
    1. Spin down fluorescein stock tube to make sure pellet is at the bottom of tube.
    2. Prepare 2x fluorescein stock solution (100  $\mu$ M) by resuspending fluorescein in 1 mL of 1xPBS.
    3. Dilute the 2x fluorescein stock solution with 1xPBS to make a 1x fluorescein solution and resulting concentration of fluorescein stock solution 50  $\mu$ M.
  - Prepare the serial dilution of fluorescein
    1. Add 100  $\mu$ l of PBS into wells A2, B2, C2, D2....A12, B12, C12, D12
    2. Add 200  $\mu$ l of fluorescein 1x stock solution into A1, B1, C1, D1
    3. Transfer 100  $\mu$ l of fluorescein stock solution from A1 into A2.
    4. Mix A2 by pipetting up and down 3x and transfer 100  $\mu$ l into A3.
    5. Mix A3 by pipetting up and down 3x and transfer 100  $\mu$ l into A4.
    6. Mix A4 by pipetting up and down 3x and transfer 100  $\mu$ l into A5.
    7. Mix A5 by pipetting up and down 3x and transfer 100  $\mu$ l into A6.
    8. Mix A6 by pipetting up and down 3x and transfer 100  $\mu$ l into A7.
    9. Mix A7 by pipetting up and down 3x and transfer 100  $\mu$ l into A8.
    10. Mix A8 by pipetting up and down 3x and transfer 100  $\mu$ l into A9.
    11. Mix A9 by pipetting up and down 3x and transfer 100  $\mu$ l into A10.
    12. Mix A10 by pipetting up and down 3x and transfer 100  $\mu$ l into A11.
    13. Mix A11 by pipetting up and down 3x and transfer 100  $\mu$ l into liquid waste.
- Take care not to continue serial dilution into column 12**
14. Repeat serial dilute for Row B 、 D 、 E
  15. Measure fluorescence of all samples in all standard measurement modes in instrument and record the data in your notebook
  16. Import data into Excel (fluorescein standard curve tab ) Sheet\_1 provided

## Data Result

uM Fluorescein	50.00	25	12.5	6.25	3.125	1.5625	0.78125	0.390625	0.195313	0.097656	0.048828	0
Replicate 1	30160	21489	12701	6126.6	3491.5	1810.5	951.09	463.12	251.25	125.75	150.38	1.592
Replicate 2	30680	21356	12790	6003.4	3094.2	2174.3	985.61	483.95	260.96	124.31	169.21	1.719
Replicate 3	30303	20001	14451	6364.6	3603.2	1903.7	940.64	474.9	258.05	131.79	153.42	1.595
Replicate 4	30239	21545	13211	6040.1	3511.5	1846.1	898.05	460.6	240.48	123.88	142.72	1.672
Arith. Mean	30345.5	21097.75	13288.25	6133.675	3425.1	1933.65	943.8475	470.6425	252.685	126.4325	153.9325	1.6445
Arith. Std.Dev.	230.5421	735.4506	806.4471	162.3825	225.897	164.965	36.0752	10.84214	9.097254	3.660094	11.1356	0.061949



## Cell Measurement

### Materials

Competent cells ( *Escherichia coli* strain DH5 $\alpha$ )

LB (Luria Bertani) media

Chloramphenicol (stock concentration 25 mg/mL dissolved in EtOH - working stock 25  $\mu$ g /mL)

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

## Pipettes

96 well plate(cell culture 96 well plate 、 tissue culture testplate)

Devices (from InterLab Measurement Kit):

1. Negative control(BBa\_R0040)
2. Positive control(J23151+B0032+E0040+B0010+B0012)
3. Test Device 1: J23101+I13504
4. Test Device 2: J23106+I13504
5. Test Device 3: J23117+I13504
6. Test Device 4: J23101+BCD2+E0040+B0015
7. Test Device 5: J23106+BCD2+E0040+B0015
8. Test Device 6: J23117+BCD2+E0040+B0015

## Method

1. Day 1 : Resuspended each plasmid in plate 7 and transform into Escherichia coli DH5 $\alpha$ .

(Transformation protocol is from iGEM)

2. Day 2 : Pick 2 colonies from each of plate and inoculate it on 5-10 mL LB medium +Chloramphenicol. Grow the cells overnight (16-18 hours) at 37°C and 225 rpm.

3. Day 3 : Set instrument to read OD600 (as OD calibration setting)and measure OD600 of the overnight cultures

4. Dilute the cultures to a target OD 600 of 0.02 in 12 ml LB medium + Chloramphenicol in 50 mL falcon tube (covered with foil to block light).

5. Incubate the cultures at 37°C and 225 rpm.

6. Take 500  $\mu$ l samples of the cultures at 0, 2, 4, and 6 hours of incubation and place samples on ice.

7. 4 replicates of 100  $\mu$ l samples were taken from each culture at 0, 2, 4, and 6 hours of incubation and placed in a 96 well plate for OD and fluorescence measurements using the setup described in **InterLab\_2017\_Plate\_Reader\_Protocol**.

## Data Result

<b>Abs600 Raw Readings:</b>									
<b>Hour 0:</b>	Neg. Contr	Pos. Contr	Device 1	Device 2	Device 3	Device 4	Device 5	Device 6	LB + Chlor (blank)
Colony 1, Replicate	0.058	0.059	0.059	0.053	0.054	0.055	0.055	0.055	0.047
Colony 1, Replicate	0.055	0.055	0.058	0.053	0.056	0.055	0.054	0.055	0.046
Colony 1, Replicate	0.055	0.053	0.056	0.053	0.057	0.055	0.055	0.06	0.047
Colony 1, Replicate	0.055	0.059	0.053	0.053	0.058	0.055	0.061	0.055	0.048
Colony 2, Replicate	0.055	0.056	0.055	0.055	0.055	0.055	0.057	0.056	0.047
Colony 2, Replicate	0.054	0.055	0.055	0.054	0.055	0.053	0.055	0.053	0.047
Colony 2, Replicate	0.055	0.06	0.058	0.055	0.055	0.294	0.055	0.054	0.043
Colony 2, Replicate	0.056	0.054	0.058	0.054	0.055	0.054	0.055	0.054	0.05
<b>Hour 2:</b>	Neg. Contr	Pos. Contr	Device 1	Device 2	Device 3	Device 4	Device 5	Device 6	LB + Chlor (blank)
Colony 1, Replicate	0.134	0.114	0.064	0.109	0.115	0.079	0.113	0.124	0.046
Colony 1, Replicate	0.114	0.105	0.063	0.104	0.12	0.079	0.107	0.122	0.046
Colony 1, Replicate	0.133	0.104	0.061	0.103	0.115	0.079	0.121	0.13	0.045
Colony 1, Replicate	0.112	0.104	0.066	0.107	0.126	0.078	0.108	0.113	0.048
Colony 2, Replicate	0.131	0.107	0.145	0.108	0.129	0.087	0.124	0.132	0.047
Colony 2, Replicate	0.117	0.104	0.064	0.104	0.116	0.06	0.099	0.116	0.047
Colony 2, Replicate	0.126	0.117	0.062	0.11	0.121	0.081	0.114	0.102	0.045
Colony 2, Replicate	0.117	0.1	0.065	0.096	0.117	0.08	0.12	0.119	0.048
<b>Hour 4:</b>	Neg. Contr	Pos. Contr	Device 1	Device 2	Device 3	Device 4	Device 5	Device 6	LB + Chlor (blank)
Colony 1, Replicate	0.202	0.166	0.112	0.195	0.191	0.145	0.182	0.188	0.047
Colony 1, Replicate	0.182	0.189	0.111	0.186	0.192	0.156	0.18	0.198	0.046
Colony 1, Replicate	0.187	0.183	0.115	0.166	0.195	0.15	0.182	0.195	0.046
Colony 1, Replicate	0.2	0.161	0.114	0.181	0.191	0.143	0.18	0.186	0.05
Colony 2, Replicate	0.178	0.161	0.176	0.183	0.189	0.154	0.188	0.197	0.048
Colony 2, Replicate	0.172	0.161	0.093	0.199	0.185	0.145	0.175	0.1	0.047
Colony 2, Replicate	0.165	0.166	0.099	0.179	0.184	0.14	0.187	0.187	0.046
Colony 2, Replicate	0.174	0.164	0.095	0.173	0.183	0.149	0.18	0.195	0.048

Fluorescence Raw Readings:										
Hour 0:	Neg. Contr	Pos. Contr	Device 1	Device 2	Device 3	Device 4	Device 5	Device 6	LB + Chlor	(blank)
Colony 1, Replicate	43.722	50.283	54.296	50.486	43.719	50.481	44.69	41.834	42.542	
Colony 1, Replicate	44.113	49.953	55.295	50.006	43.485	51.164	43.964	42.306	42.226	
Colony 1, Replicate	42.607	42.155	53.664	47.694	42.699	49.304	42.926	35.968	40.172	
Colony 1, Replicate	43.162	49.806	54.932	51	40.856	52.504	42.872	42.199	41.346	
Colony 2, Replicate	41.959	49.301	55.301	48.672	42.524	31.984	46.518	40.238	41.802	
Colony 2, Replicate	42.621	50.206	55.611	51.667	44.367	51.886	46.301	41.874	45.276	
Colony 2, Replicate	41.745	42.592	55.569	50.533	41.799	45.105	45.336	38.526	44.066	
Colony 2, Replicate	41.812	46.064	54.252	51.703	43.679	188.65	44.951	40.653	20.088	
Hour 2:	Neg. Contr	Pos. Contr	Device 1	Device 2	Device 3	Device 4	Device 5	Device 6	LB + Chlor	(blank)
Colony 1, Replicate	45.544	90.822	69.566	95.206	46.304	75.76	59.712	43.132	42.908	
Colony 1, Replicate	44.043	92.065	68.049	101	42.948	76.325	60.074	43.32	141.893	
Colony 1, Replicate	42.586	84.106	64.22	90.21	43.287	70.623	53.921	41.427	40.675	
Colony 1, Replicate	45.239	81.456	66.229	92.75	42.016	72.339	58.287	41.248	35.95	
Colony 2, Replicate	43.658	88.272	68.371	83.823	44.315	72.576	60.832	41.542	42.597	
Colony 2, Replicate	44.418	94.467	73.328	98.281	45.17	78.449	61.75	43.734	44.432	
Colony 2, Replicate	44.773	83.83	69.888	88.61	42.769	46.565	47.184	39.088	41.566	
Colony 2, Replicate	44.204	93.152	70.715	88.317	43.03	71.006	54.894	33.013	30.39	
Hour 4:	Neg. Contr	Pos. Contr	Device 1	Device 2	Device 3	Device 4	Device 5	Device 6	LB + Chlor	(blank)
Colony 1, Replicate	48.026	128.74	97.516	160.32	49.625	110.99	70.233	47.197	42.648	
Colony 1, Replicate	45.911	125.73	97.161	164.6	47.738	107.2	67.064	45.697	44.36	
Colony 1, Replicate	44.665	132.25	92.394	194.44	47.097	104.31	67.365	45.774	43.131	
Colony 1, Replicate	46.864	132.27	96.165	152.57	49.596	111.4	69.493	47.683	44.377	
Colony 2, Replicate	43.843	128.75	99.212	145.84	45.843	106.31	65.994	44.736	41.269	
Colony 2, Replicate	46.915	135.67	104.14	155.06	53.288	114.79	70.465	47.224	44.254	
Colony 2, Replicate	44.53	130.38	92.773	150.31	47.538	102.76	63.91	26.013	43.513	
Colony 2, Replicate	41.172	127.63	101.06	147.96	46.256	97.558	65.731	44.064	36.329	
Hour 6:	Neg. Contr	Pos. Contr	Device 1	Device 2	Device 3	Device 4	Device 5	Device 6	LB + Chlor	(blank)
Colony 1, Replicate	47.767	138.58	105.59	164.44	47.612	127.72	65.606	47.847	42.309	
Colony 1, Replicate	50.915	149.17	111.05	178.97	49.814	130.92	69.479	48.27	44.296	
Colony 1, Replicate	45.694	143.05	109.55	169.34	47.703	138.49	67.133	44.318	43.126	
Colony 1, Replicate	49.766	152.24	113.11	178.23	49.309	137.42	70.29	47.719	35.699	
Colony 2, Replicate	45.428	159.4	125.45	168.49	46.752	124.12	67.705	44.97	41.697	
Colony 2, Replicate	48.873	165.21	131.56	178.85	49.177	131.57	66.719	49.331	43.459	
Colony 2, Replicate	42.278	168.81	136.3	193.27	48.693	139.27	67.406	42.234	42.523	
Colony 2, Replicate	48.573	174.69	139.64	190.52	49.433	142.4	70.511	48.279	27.67	