Automated Serial Dilution

UC Davis iGEM 2019

Repeat this Protocol Three Times with both your Opentrons OT-2 Robot and an iGEM Team Member

To best standardize this protocol, we have based it upon the 2019 iGEM Fluorescein and Abs600 Calibration Protocols. We invite you to perform serial dilutions using both your Opentrons OT-2 robot and a team member to determine the accuracy of the Opentrons OT-2.

Tips:

- Be sure to set the robot to calibrate to the bottom of well A1 for each labware, by default the robot calibrates to the top. This setting can be found on the "Robot" page of the Opentrons application.
- When calibrating the OT2 to the bottom of the instruments, make sure to go all the way to the bottom. If you cannot see the bottom of the 96 well plate, try marking a pipette tip with the appropriate height and lining it up with the OT2 while calibrating.**
- To avoid contaminating the pipette, **do not fill 15mL tubes past 7mL****

Materials:

- iGEM Measurement kit
- Opentrons OT2 Robot
    - Opentons p300 pipette installed on the RIGHT
    - Opentrons p300 tips
    - Opentrons 15mL tube rack (4 in 1 Rack)
    - Opentrons 96 well flat plate
- 1XPBS (Phosphate-buffered saline)
- Fluorescein kit tube
- p300 Pipette
- 15 mL conical tubes
- ddH2O (double distilled water)

Protocol for Making 1X PBS

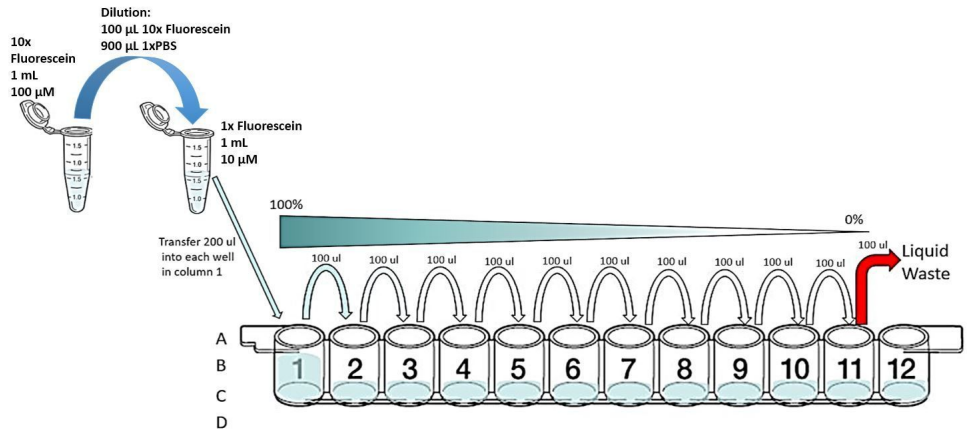
1. Dissolve the following in 800 mL DI water
2. 8 g NaCl
3. 0.2 g KCl
4. 1.4 g Na2 HPO4
5. 0.24 g KH2 PO4
6. Adjust the pH to 7.4 using HCl.
7. Fill to 1 L
8. Filter (0.22 uM) Sterilize

Fluorescein:

Manual

1. Spin down fluorescein kit tube to make sure reagent is at the bottom of tube
2. Prepare 10x fluorescein stock solution (100uM) **by resuspending fluorescein in 1mL of 1X PBS**
    a. **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.
3. Dilute the 10X fluorescein stock solution with 1X PBS to make a 1X fluorescein solution at 10 µM: **100 µL of 10X fluorescein stock into 900 µL 1X PBS** into a 15mL tube.

4. 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 1X PBS in columns 2 to 12.
    a. You will perform a serial dilution by consecutively transferring 100 µl from column to column with good mixing.



(iGEM Protocol)

5. Add 100 µl of 1X PBS into wells A2, B2, C2, D2....A12, B12, C12, D12
6. Add 200 µl of fluorescein 1X stock solution into A1, B1, C1, D1
7. Transfer 100 µl of fluorescein stock solution from A1 into A2
8. Mix A2 by pipetting up and down 3x and transfer 100 µl into A3
9. Mix A3 by pipetting up and down 3x and transfer 100 µl into A4
10. Mix A4 by pipetting up and down 3x and transfer 100 µl into A5
11. Mix A5 by pipetting up and down 3x and transfer 100 µl into A6
12. Mix A6 by pipetting up and down 3x and transfer 100 µl into A7
13. Mix A7 by pipetting up and down 3x and transfer 100 µl into A8
14. Mix A8 by pipetting up and down 3x and transfer 100 µl into A9
15. Mix A9 by pipetting up and down 3x and transfer 100 µl into A10
16. Mix A10 by pipetting up and down 3x and transfer 100 µl into A11

17. Mix A11 by pipetting up and down 3x and transfer 100 µl into liquid waste (**Do not perform a serial dilution into column 12**).
18. Repeat dilution series for rows B, C, D

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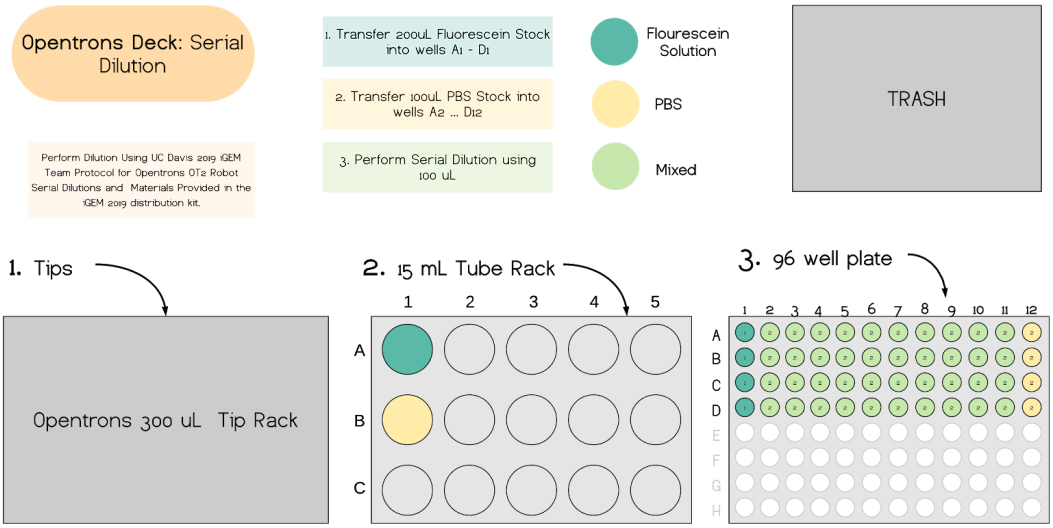
19. Repeat Steps 1-3.
20. Place 1mL Fluorescein Solution into A1 of your Opentrons OT2 15mL tube rack.
21. Place 5 mL 1X PBS into B1.



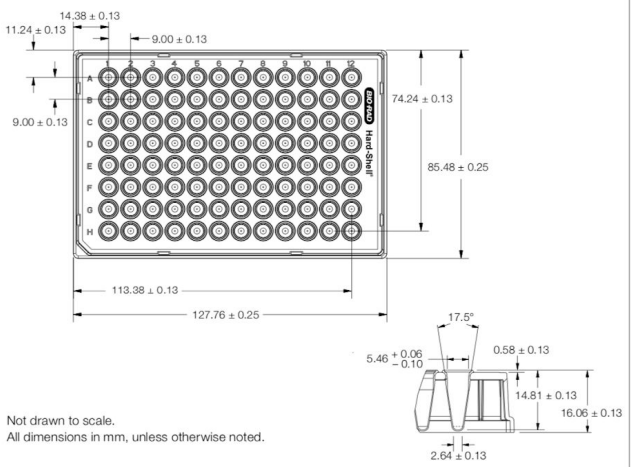
22. Calibrate your OT2 to the BOTTOM of your labware and using the following deck layout:

23. Using our Opentrons OT2 Protocol, perform a serial dilution: [iGEM 2019 Serial Dilutions Test]

Plate Reader

24. Measure Plates using a plate reader

Plate Dimensions: Bio-Rad Hard-Shell Low Profile 96-Well Skirted PCR Plates (from Opentrons OT2 Setup)



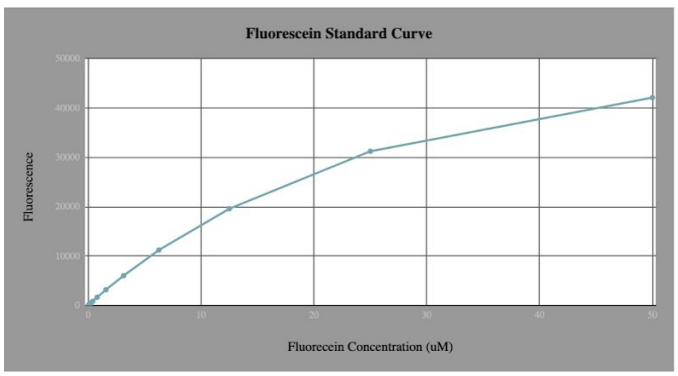
Microplate Dimensions	Hard-Shell Low-Profile 96-Well Skirted PCR Plates (HSP-9xxx)
Length at base plane	127.76 mm
Width at base plane	85.48 mm
Height overall	16.06 mm
Well depth	14.81 mm
Well diameter at opening	5.46 mm
Well diameter at bottom of conical section	2.64 mm
Well volume	200 µl
Well spacing	9.00 mm
Well angle	17.5°
Well offset	
Left edge to well A1	14.38 mm
Top edge to well A1	11.24 mm
Left edge to H12	113.38 mm
Top edge to H12	74.24 mm

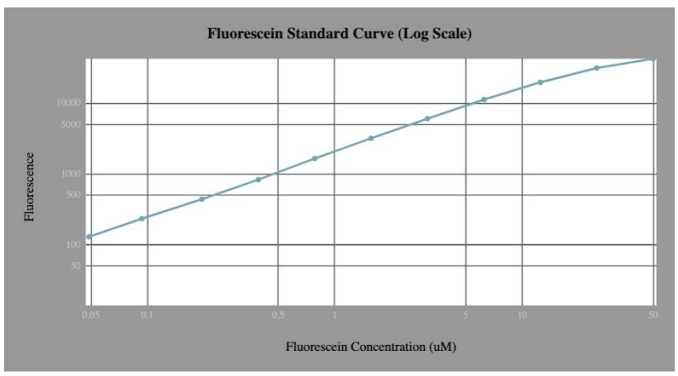
Plate Reader Settings:	
Excitation	485 nm
Emission	535 nm
Excitation Bandwidth**	5 nm
Emission Bandwidth **	5 nm
Optics	Top Reading
Gain	Optimal (automatic)
Number of Flashes	50
Flash Frequency **	400 Hz
Integration Time	20 us
Lag Time	0 us
Settle Time	0 ms
Part of Plate	ALL
Temperature **	26.8 degrees Celsius

** Some Settings are not adjustable on all plate readers, if this is the case for your plate reader, ignore these sections **

25. Record Data
26. Put information into Spreadsheet provided:
    IGEM_2019_Data_Analysis_Template_Fluorescence_Standard_Curve_Protocol_v1

uM Fluorescein	50	25	12.5	6.25	3.125	1.5625	0.78215	0.391075	0.195313	0.093513	0.048828	0
OT2-1	41521	30938	19437	10982	5928	3095	1609	830	427	230	129	15
OT2-2	42351	31235	19619	11186	5927	3195	1682	875	460	244	141	19
OT2-3	42437	31411	19469	11270	6079	3164	1664	770	430	227	127	15
OT2-4	42216	31422	19899	11433	6163	3274	1654	856	446	236	123	7
Arith. Mean	42131.25	31251.5	19606	11217.75	6024.25	3182	1652.25	832.75	440.75	234.25	130	14
Arith. Std. Dev.	416.87758	225.8797615	210.8301054	187.6599318	116.8628113	74.22039253	31.0738368	45.71925196	15.30522787	7.5	7.745966692	5.033222957





Abs600 (OD) :

Manual

1. Obtain the tube labeled “Silica Beads” from the Measurement Kit and vortex vigorously for 30 seconds.
2. Immediately pipette 100 uL microspheres into 15 mL tube
3. Add 900 uL of ddH2O to the microspheres
4. Vortex well: This is your Microsphere Stock Solution
5. Perform a Serial Dilution using the same protocol as for Fluorescein (Steps 1- 18). Using ddH2O in the place of PBS and Microphere Stock Solution instead of Fluorescein.
    a. Vortex the Microsphere Stock immediately before adding 200 uL to A1:D1.

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6. Repeat Steps 1-3.
7. Place 1mL Microsphere Stock Solution into A1 of your Opentrons OT2 15mL tube rack.
8. Place 5 mL 1X ddH2O into B1.
9. Using the same Opentrons Protocol as above, perform a Serial Dilution using your Opentrons OT-2 robot.

Plate Reader

10. Measure OD600 of all samples in instrument

Plate Reader Settings:	
Measurement	600 nm
Number of Flashes	25
Lag Time	0 us
Settle Time	0 ms
Part of Plate	ALL
Temperature **	26.8 degrees Celsius

11. Record Data
12. Put information into Spreadsheet provided: IGEM_2019_Data_Analysis_Template_Particle_Standard_Curve_Protocol_v1