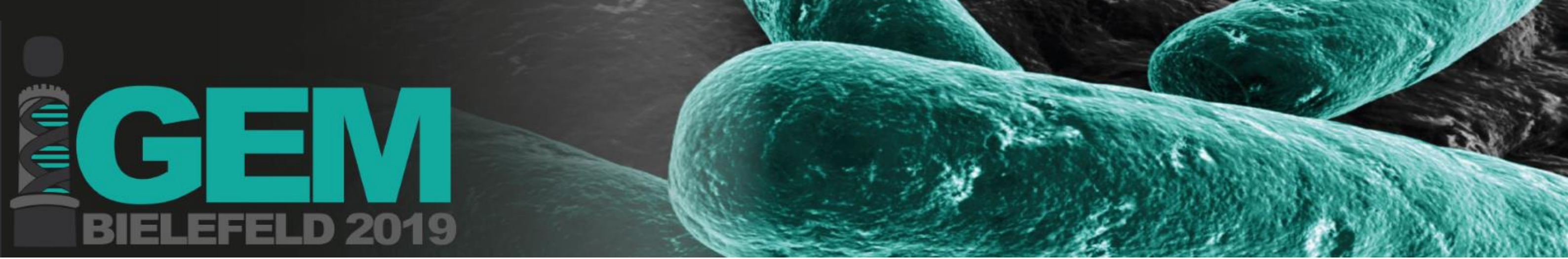


Protocol: Texas Red as a Standard to measure absolute mCherry fluorescence

In order to compare mCherry fluorescence measurements with each other, regardless of the device, the time of measurement or the person conducting the experiments, a standardization of the data has to be done.

Within the iGEM community this has already been done for GFP over the last few years by establishing Fluorescein as a reference. The experiments have been conducted in many labs and over several years to ensure a sound standardized method.

However, for many projects, GFP might not be the right – or the only – choice. Therefore, standardization needs to be available for more fluorescent proteins.

This is why we are suggesting Texas Red as a reference for all mCherry measurements conducted within the iGEM competition – and to establish this, we need the support of as many 2019 iGEM teams as possible. If you are working with mCherry, feel free to use the following standard series to get better and more comparable results – and send them to us, to become a part of our measurement wiki page. You will also receive an amazing button for your wiki to post on your collaboration page! We're grateful for all the data we can get, so in which ever application you're using mCherry, let us know! Looking forward to collaborating with teams from all around the world!

Materials you need to provide:

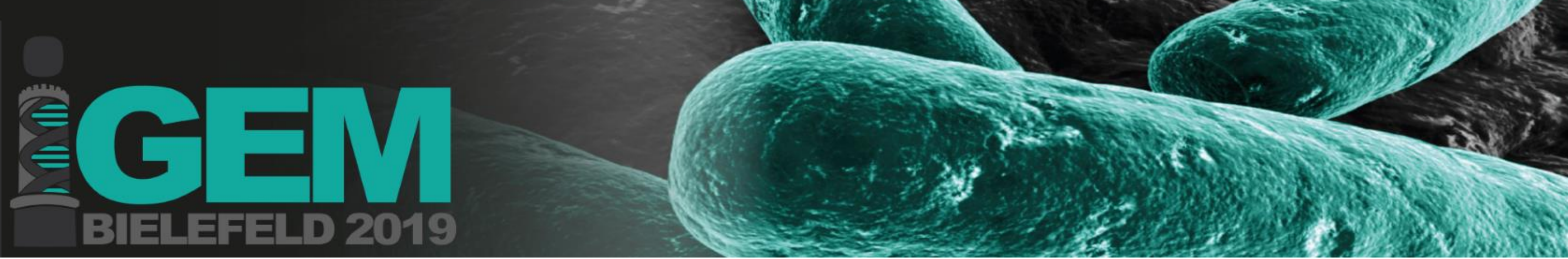
A plate reader for fluorescence measurements

96 well-plate (ideal: black with a clear flat bottom)

PBS

Texas Red (if you are having issues getting hold of that: get in touch!)

Optional: LB-Cm, 50 ml tubes (opaque or wrapped in tin foil), 37°C shaking incubator that can hold those

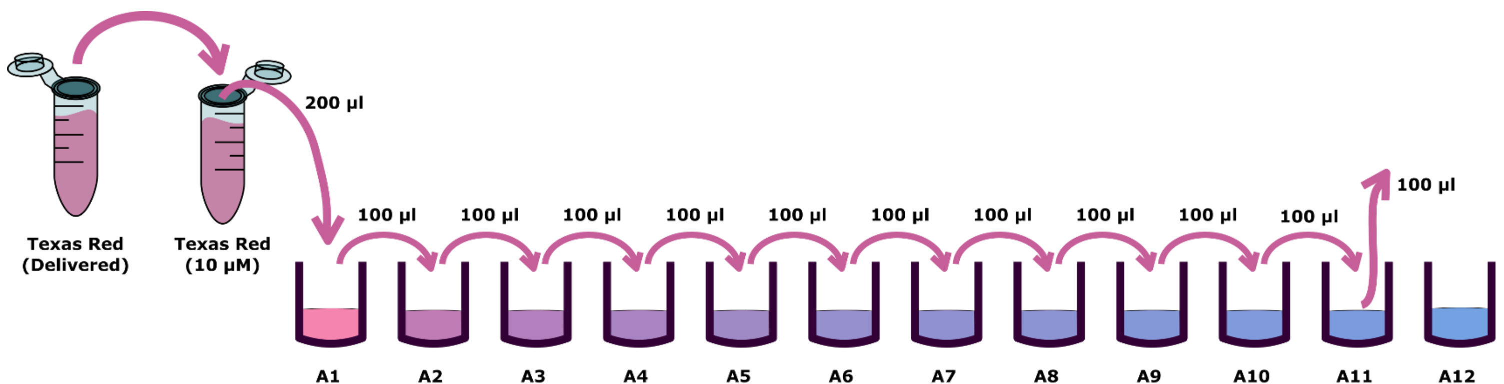


Protocol:

1. Prepare a stock solution of Texas Red (100 µM) with 1x PBS in a 1.5 ml opaque tube.
2. Dilute the 10x Texas Red stock solution with 1x PBS to make a 1x Texas Red solution with a concentration of 10 µM:

   100 µl 10x stock + 900 µl 1x PBS

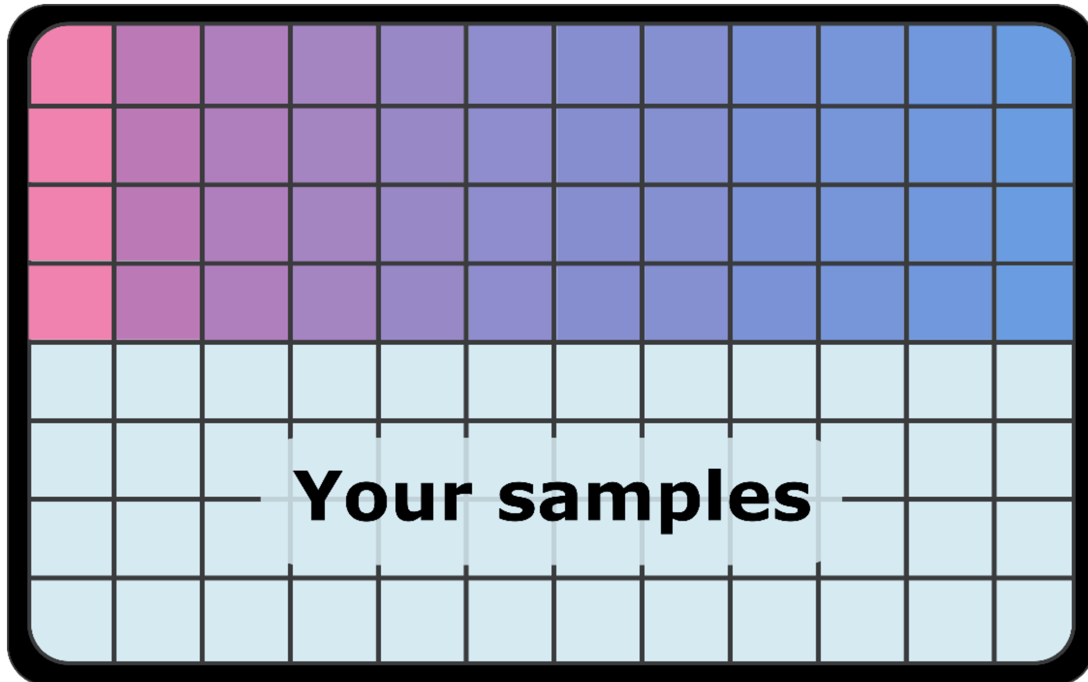
3. Prepare a serial dilution in a plate in line 1-4, column 1-11. Column 12 contains PBS buffer only:
   a. Add 100 µl 1x PBS to all wells A2-A11, B2-B11, C2-C11 and D2-C11.
   b. Add 200 µl 1x Texas Red into A1.
   c. Transfer 100 µl from A1 to A2. Mix well.
   d. Transfer 100 µl from A2 to A3. Mix well.
   Continue with these steps until you reach column 11. Discard 100 µl of column 11 – Do not transfer anything from column 11 to column 12!



Repeat for B, C and D.

<u>Make sure to work precisely and quickly. Try avoiding light by storing the plate in the dark during the experiments as well as you can. We do that by putting them into Styrofoam boxes with lids whenever possible to prevent photodegradation of the Texas Red.</u>

This is what your plate should look like after pipetting all dilution series:



4. Measure the fluorescence of your plate in your plate reader – make sure that any automatic gain settings are turned off.

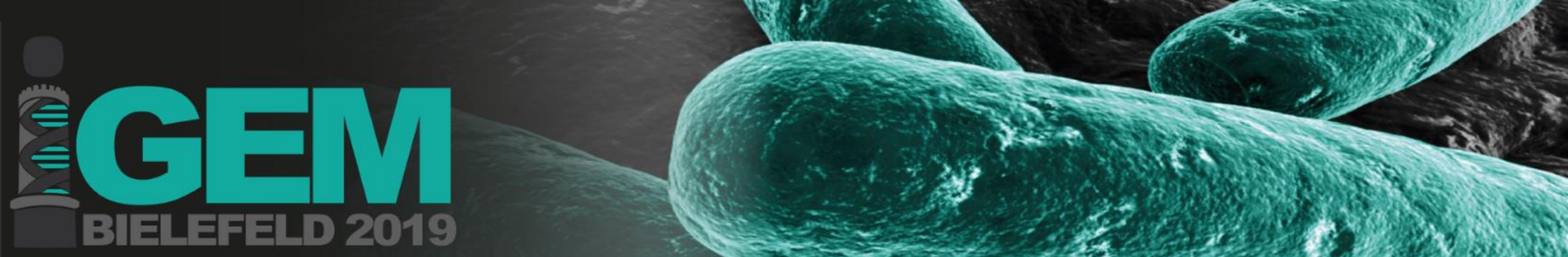
   Set Excitation to: 570 nm
   Set Emission to: 610 nm

5. Add the data your plate reader recorded to the sheet "Texas Red Standard Curve" to get the equivalent Texas Red concentration for the fluorescence you recorded for your mCherry samples!
6. Send us the data you recorded to receive the button for your wiki!

If your plate reader can record OD_{600}-data, feel free to repeat the experiment with a 1x stock solution of microspheres provided in the iGEM-Measurement kit. To get the 1x stock solution, you need to mix 100 µl of the Kit's microsphere suspension with 900 µl ddH_2O.

By doing so, you can determine the fluorescence per particle, enabling you to compare results from different cultures even better.

Check out this protocol if you want to learn more about using the microspheres.



If you want to help us out even further, gaining a special place in our hearts and on our wiki, compare the fluorescence of the following strains against Texas Red:

E. coli ER2566 (pSB1C3-Bba_J23**104**-mCherry-His) (=104)

E. coli ER2566 (pSB1C3-Bba_J23**114**-mCherry-His) (=114)

E. coli ER2566 (pSB1C3-**P8Prom**-mCherry-His) (=P8)

We would be happy to send you the plasmids or the strains to enable you to do this experiment.

Protocol:

Day one:

1. Prepare three overnight cultures, one of each strain and put an additional flask/tube with LB-Cm as a negative control in the shaking incubator set to 37°C.

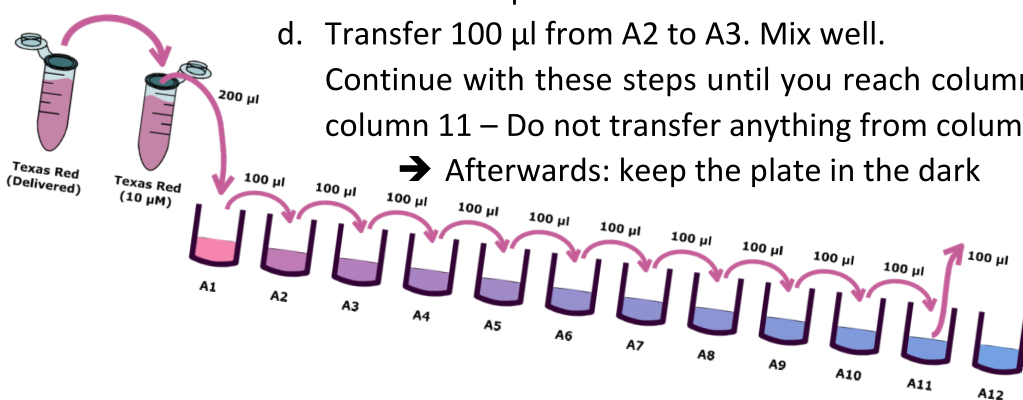
Day two:

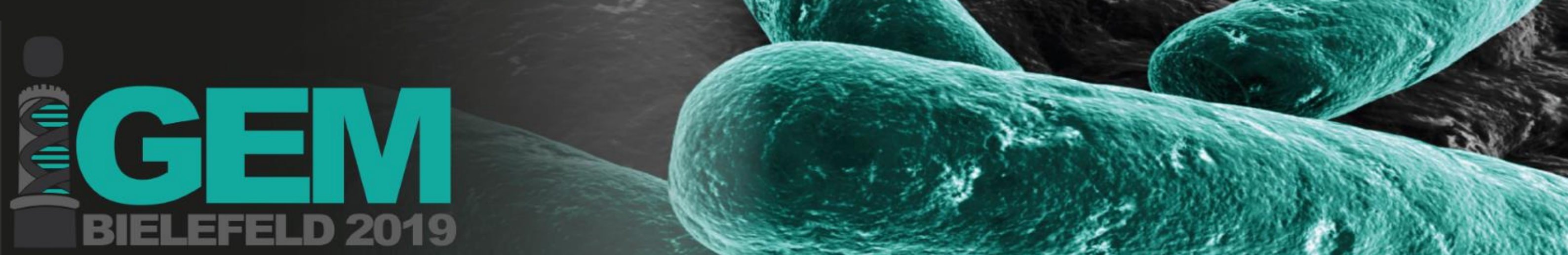
2. Measure the OD_{600} of your overnight cultures. Set up three dilutions of each culture with an OD_{600} of 0.02 in a final volume of 10 ml in a darkened 50 ml tube (either by design or by tin foil). This should result in 9 50 ml tubes filled with 10 ml culture each.
   Remove 500 µl from each tube, store in 1.5 ml tubes on ice and in the dark. Place the 50 ml tubes with the left-over cultures in a shaking incubator and incubate for 6h at 37°C.

   After 6 h:
3. Prepare a stock solution of Texas Red (100 µM) with 1x PBS in an opaque tube.
4. Dilute the 10x Texas Red stock solution with 1x PBS to make a 1x Texas Red solution with a concentration of 10 µM:

   100 µl 10x stock + 900 µl 1x PBSPrepare a serial dilution in a plate in line 1-3, column 1-11. Column 12 contains PBS buffer only:

   a. Add 100 µl 1x PBS to all wells A2-A11, B2-B11 and C2-C11.
   b. Add 200 µl 1x Texas Red into A1.
   c. Transfer 100 µl from A1 to A2. Mix well.
   d. Transfer 100 µl from A2 to A3. Mix well.
   Continue with these steps until you reach column 11. Discard 100 µl of column 11 – Do not transfer anything from column 11 to column 12!
   ➔ Afterwards: keep the plate in the dark





Take your cultures out of the incubator and place them on ice. Determine the OD_{600} of each. Transfer 3x 100 µl of your 6h cultures as well as 3x 100 µl of your start cultures (1.5 ml tubes) (OD_{600}=0.02) to the plate. Your setup should look like this:

Texas Red

104 A	104 A	104 A	104 A	104 A	104 A	114 C	114 C	114 C	114 C	114 C	114 C
104 B	104 B	104 B	104 B	104 B	104 B	P8 A	P8 A	P8 A	P8 A	P8 A	P8 A
104 C	104 C	104 C	104 C	104 C	104 C	P8 B	P8 B	P8 B	P8 B	P8 B	P8 B
114 A	114 A	114 A	114 A	114 A	114 A	P8 C	P8 C	P8 C	P8 C	P8 C	P8 C
114 B	114 B	114 B	114 B	114 B	114 B	LB-Cm	LB-Cm	LB-Cm	LB-Cm	LB-Cm	LB-Cm

6h 0h

Measure the fluorescence of your plate in your plate reader – make sure that any automatic gain settings are turned off.

Set Excitation to: 570 nm
Set Emission to: 610 nm

If your plate reader is able to record OD_{600}, feel free to convert the values you recorded to fluorescence per particle.

Afterwards, you can add the values to the provided excel sheet and it will calculate the equivalent of Texas Red to the fluorescence recorded for mCherry. Send us your filled out excel file and get your button as a response!

Thank you for participating in our small interlab study! We appreciate your work and hope, we added something valuable to your project!

iGEM Bielefeld-CeBiTec 2019

All protocols adjusted from the interlab study 2018. Thanks to the measurement committee for providing us with all the information we need.