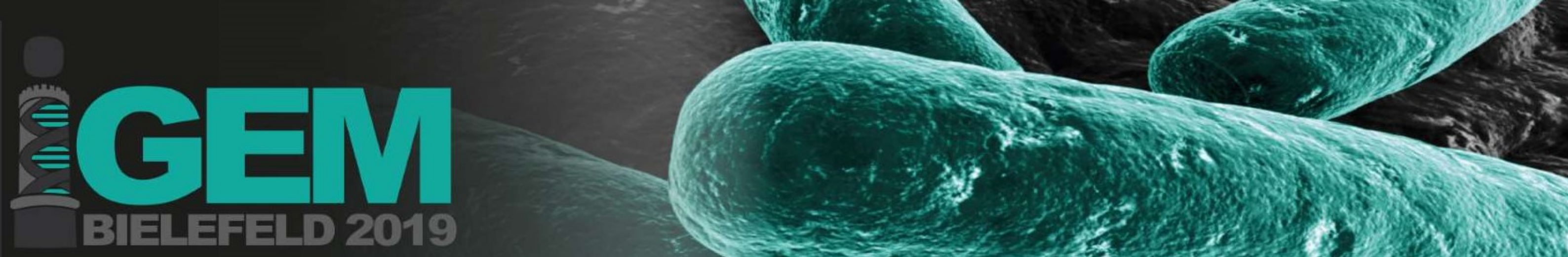


Protocol: Texas Red as a Standard to measure absolute mCherry fluorescence

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

Within the iGEM community this has been done for GFP in the last few years by establishing Fluorescein as a reference. This has been done in many labs and over several years to ensure a sound standard 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. We're thankful for all the data we can get, so in which ever application you're using mCherry, let us know! Looking forward to all the collaborations with teams all around the world!

Be aware that you should conduct all OD-measurements at a wavelength of 660 nm rather than 600 if your cells express a red fluorescent protein, since the protein also absorbs light at 600 nm and you want to avoid any interference.

Materials you need to provide:

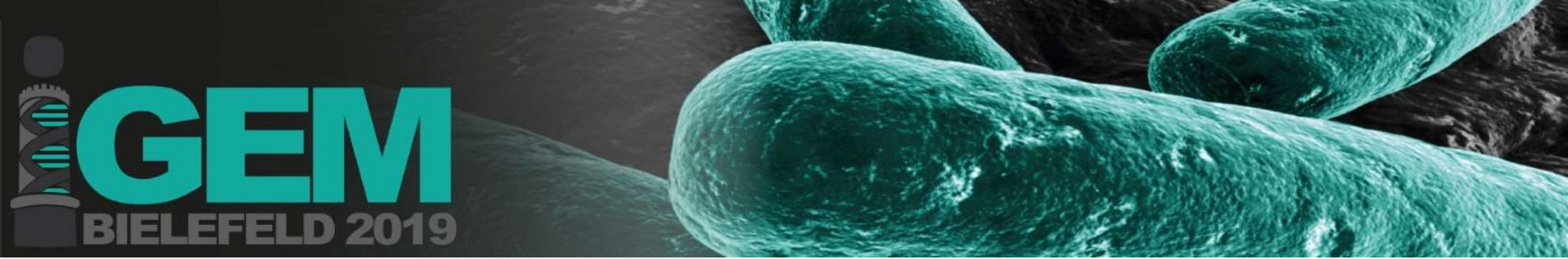
A plate reader for fluorescence measurements

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, 1.5 ml tubes), 37°C shaker incubator

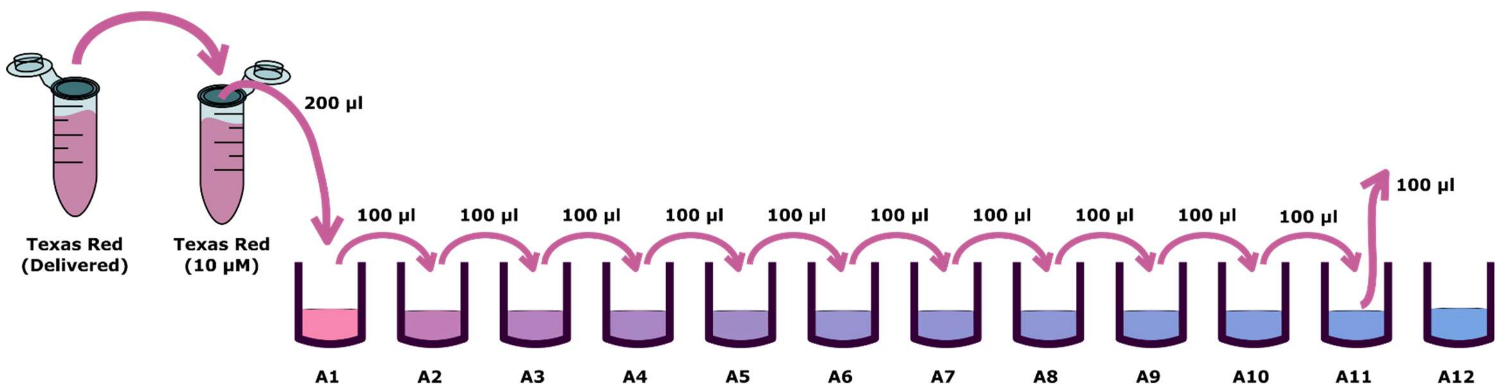


Protocol:

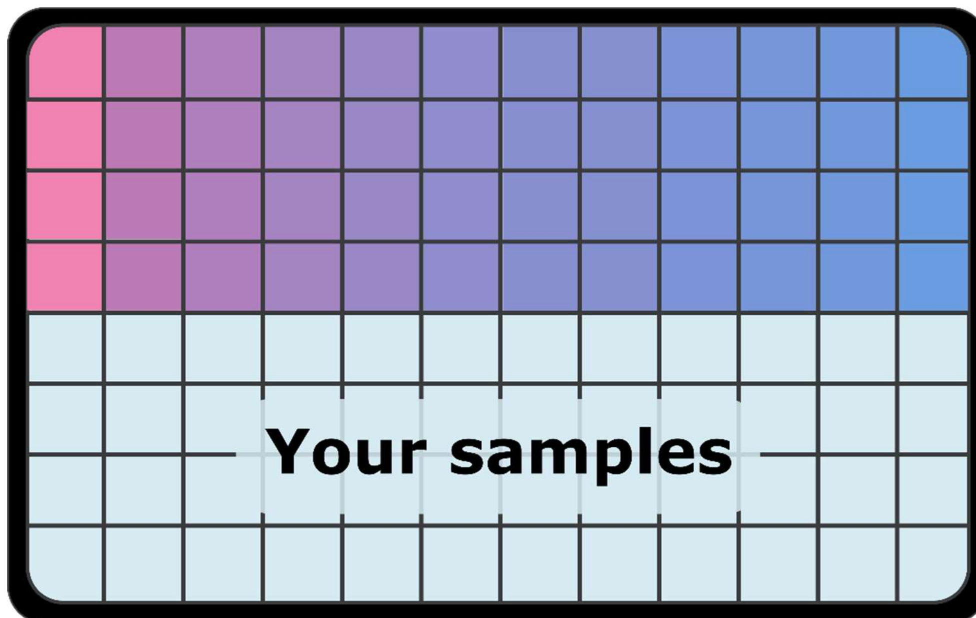
1. Prepare a stock solution of Texas Red (100 µM) with 1x PBS in an opaque tube.
2. Dilute the 10x Texas Red stock solution with 1x PBS to make a 1x Texas Red solution with a concentration of 10 µM:

   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.

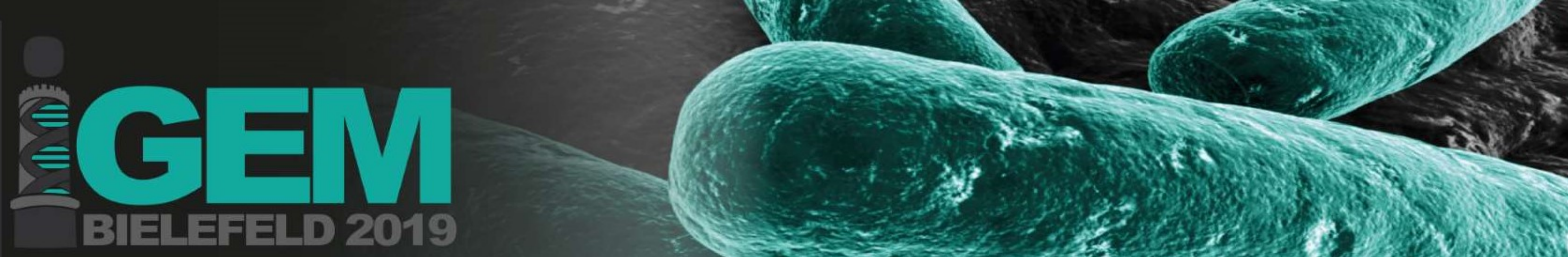


This is what your plate should look like after pipetting all dilution series. Make sure to work precisely and quickly. Store the plate in the dark as often and long as possible. We do that by putting them into Styrofoam boxes with lids whenever possible to prevent photodegradation of the Texas red.

4. Measure the fluorescence of your plate in your plate reader – make sure that any automatic gain setting is turned off.
   Set Excitation to: 570 nm
   Set Emission to: 610 nm

If your plate reader is able to record OD_{660}-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 this, you can convert particle count to mCherry, enabling you to compare results from different cultures even better.



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_J23104-mCherry-His)

E. coli ER2566 (pSB1C3-Bba_J23114-mCherry-His)

E. coli ER2566 (pSB1C3-P8Prom-mCherry-His)

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

<u>Protocol</u>:

Day one:

1. Prepare three overnight cultures, one of each strain and put an additional flask/tube with LB-Cm in the shaker.

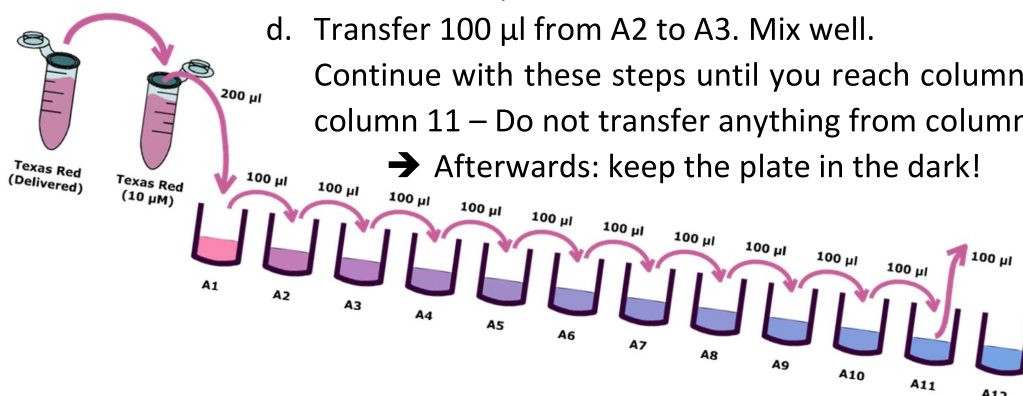
Day two:

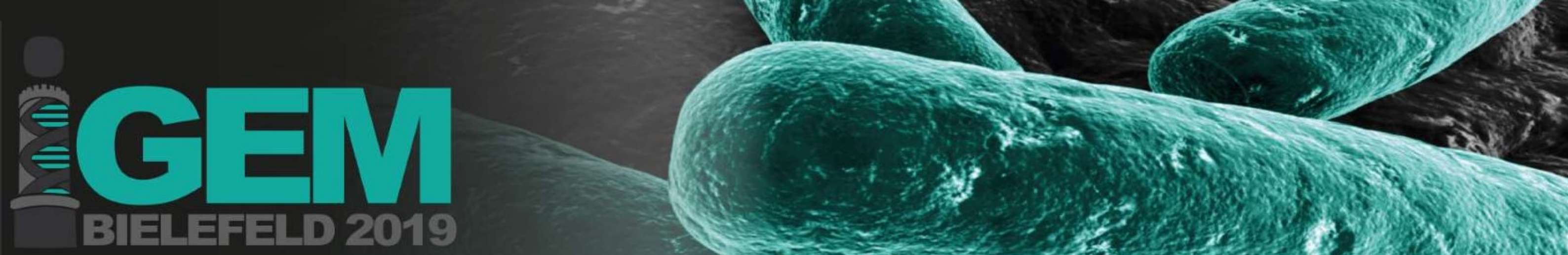
2. Measure the OD_{660} of your overnight cultures. Dilute each culture <u>three times</u> to an OD_{660} of 0.02 in a final volume of 10 ml in a darkened 50 ml tube (either by design or by tin foil). Remove 500 µl, store in a 1.5 ml tube on ice and in the dark.
   Place the cultures in the 50 ml tubes in a shaker 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, 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!
   ➔ Afterwards: keep the plate in the dark!





Take your cultures out of the incubator and place them on ice. Determine the OD_{660} of each. Transfer 3x 100 µl of your 6h cultures as well as 3x 100 µl of your start cultures (OD_{660}=0.02) to the well 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 setting is turned off.

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

If your plate reader is able to record OD_{660}, feel free to convert the OD_{660} values you recorded to fluorescence per particle.

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

iGEM Bielefeld-CeBiTec 2019