Technische Universiteit
Eindhoven
University of Technology

iGEM TU/e 2015
Biomedical Engineering

Eindhoven University of Technology
Room: Ceres 0.04
Den Dolech 2, 5612 AZ Eindhoven
The Netherlands
Tel. no. +31 50 247 55 59
2015.igem.org/Team:TU_Eindhoven

Measuring (bio)luminescence and fluorescence

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Measuring (bio)luminescense and fluorescence

1 Preparation samples

Estimated bench time: 20 minutes
Estimated total time: 20 minutes
Purpose: Prepare the cells with expressed proteins for bioluminescence or fluorescence measurement.

1.1 Materials

- Bacterial cells with expressed proteins
- Cell density meter (OD600)
- Cuvette
- dH_2O
- Eppendorf tubes
- Falcon tube
- MiniSpin Centrifuge
- PBS
- Pipette and tips
- Tabletop Centrifuge

1.2 Setup & Protocol

- The cells are ready from protein expression and need to be washed.
- Spin down the cells for 15 minutes at 3,000 g. Weight balance well.
- Discard supernatant in a Falcon tube.
- Add 1 ml PBS and resuspend by pipetting up and down and transfer it to an Eppendorf tube.
- Spin down the cells for 1 minute at 13,400 rpm.
- Discard supernatant.
- Add 1 ml PBS to an Eppendorf tube.
- Make a blank OD600 measurement by mixing 950 µl dH_2O with 50 µl PBS in a cuvette.
- Measure the OD of the sample by mixing 950 µl dH_2O with 50 µl bacterial culture. If the OD is lower than 1 continue, else dilute the sample till the OD is below 1.
- Make the following dilution series for the cells by using PBS (Make sure you have at least half the volume of one well):
    - 5x diluted
    - 50x diluted
    - 500x diluted
    - 5,000x diluted

2 Measuring (bio)luminescence

Estimated bench time: 20 minutes
Estimated total time: 1 hour and 20 minutes
Purpose: Analyses of the (bio)luminescence of the expressed proteins.

2.1 Materials

- 384 White wells plate
- Bacterial cells with expressed proteins (different dilutions)
- Cary Eclipse Fluorescence Spectrophotometer
- Cell density meter (OD600)
- Cuvette
- dH_2O
- Ethanol (70%)
- PBS
- Pipette and tips
- Substrate for proteins (for Nanoluc 100x Promega Nano-Glow)
- Tecan infinite F500 plate-reader

2.2 Setup & Protocol

- Fill the 384 white wells plate with the volumes according to the table below. Start with adding the given volumes of PBS, followed by the cells and mixing them by pipetting slowly up and down. Let it incubate for 20-30 minutes and complete by adding 1 µl substrate to each well.

Well	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**
Dilution	**10,000 x**	**1,000 x**	**100 x**	**10 x**	**2 x**	**0 x**
Cells	$X^1/2$	$X^1/2$	$X^1/2$	$X^1/2$	$X^1/2$	X^1-1
Substrate	1 µl	1 µl	1 µl	1 µl	1 µl	1 µl
PBS	$X^1/2$-1	$X^1/2$-1	$X^1/2$-1	$X^1/2$-1	$X^1/2$-1	
Total volume	X^1	X^1	X^1	X^1	X^1	X^1

- For the measurement of bioluminescence, insert the plate into the Tecan Infinite F500 plate-reader.
- Select the wells that contain the samples.
- Select Luminescence Dual Color with the filters Green and Magenta to measure the activity of mNeonGreen and NanoLuc.
- Select the Kinetic Cycle and take 60 cycles and select the Kinetic Interval to measure for one hour.
- When all the settings are chosen, start the measurement.

It is also possible to measure the bioluminescence spectrum of one concentration of cells, but in that case it is recommended to use the Cary Eclipse Fluorescence Spectrophotometer and preform the following steps instead.

- Take a cuvette (volume: 200 µl) and first clean it with dH_2O and 70% ethanol.
- Fill the cuvette with 199 µl cells of the chosen dilution and 1 µl substrate.
- Open the program and select the following parameters:
  - Select the (Bio)luminescence measurement and choose a gate time[2] of 200 ms.
  - Choose a wavelength of 400-600 nm.
  - Choose an emission slit[2] of 20 nm.
  - Select the correct multicell holder.
  - Select Autostore: storage on prompt at start.

[1] The total volume of each well is dependent on the chosen wells plate.
[2] By varying the slit and the gate time, the measured signal can be enhanced.

  - Select ASCII for autoconvert.
  - Select multiple cycles if a measurement in time is desired.
- Start the measurement.

3 Measuring fluorescence

Estimated bench time: 30 minutes
Estimated total time: 30 minutes
Purpose: Analyses of the bioluminescence of the expressed proteins.

3.1 Materials

- 384 Black wells plate
- Bacterial cells with expressed proteins (different dilutions)
- Cary Eclipse Fluorescence Spectrophotometer
- PBS
- Pipette and tips
- Substrate for proteins (for Nanoluc 100x Promega Nano-Glow)
- Tecan Safire2 plate-reader

3.2 Setup & Protocol

- Fill the 384 black wells plate with the volumes according to the table below. Start with adding the given volumes of PBS, followed by the cells and mixing them by pipetting slowly up and down. Let it incubate for 20-30 minutes and complete by adding 1 µl substrate to each well.

Well	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**
Dilution	**10,000 x**	**1,000 x**	**100 x**	**10 x**	**2 x**	**0 x**
Cells	$X^3/2$	$X^3/2$	$X^3/2$	$X^3/2$	$X^3/2$	X^3-1
Substrate	1 µl	1 µl	1 µl	1 µl	1 µl	1 µl
PBS	$X^3/2$-1	$X^3/2$-1	$X^3/2$-1	$X^3/2$-1	$X^3/2$-1	
Total volume	X^3	X^3	X^3	X^3	X^3	X^3

- For the measurement of fluorescence, open the program (Excel-file) of the Tecan Safire2 plate-reader and edit the measurement parameters.
  - Select the fluorescence measurement.
  - Plate: select the plate that is used and the wells that contain the samples.
  - Wavelength: select the emission scan with an excitation at 480 nm and emission at 500 – 600 nm.
  - Nr. of reads: select 10.
  - Select endpoints measurement as only one measurement is being done.
- Insert the plate into the Tecan Safire2 plate-reader and start the measurement.

[3] The total volume of each well is dependent on the chosen wells plate.