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GFP-RFP plate reader assay

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ABSTRACT

Fluorescence measurements of Green fluorescent protein (GFP) and/or Red fluorescent protein (RFP) in *Escherichia coli.*

MATERIALS

NAME	CATALOG #	VENDOR
96-well plate, flat bottom, tissue culture treated, black wall with clear bottom	3904	Fisher Scientific
Microplate Reader Synergy Mx	View	
50 ml Falcon tube	View	

media preparations:

1 **Prepare the following media and autoclave them according to standard procedures.**

M9TG Media:

Reagent	Amount to add for 1L	Final concentration (1×)
M9 salts	1x	1x
Tryptone	10 g	10 g/L
Glycerol	5 g	5 g/L

PBS buffer:

Reagent	Amount to add for 1L	Final concentration (1×)
NaCl	8 g	137 mM
KCl	0.2 g	2.7 mM
Na2HPO4	1.44 g	10 mM
KH2PO4	0.24 g	1.8 mM

Plate reader protocol:

2 **Set the following variables for the BioTek Synergy MX Microplate Reader:**

- Set Temperature: 37°C

Preheat before moving to next step

- Shake: Medium, 0:30 (MM:SS)

- Read OD600:
  Absorbance Endpoint of full plate
  Wavelengths:600
  Read Speed: Normal,Delay: 100 msec, Measurements/Data Point: 8

- Read GFP:
  Fluorescence Endpoint of full plate
  - Filter Set 1:
    Excitation: 485/9,0,Emission: 512/9,0
    Optics: Bottom,Gain: 50

  - Filter Set 2:
    Excitation: 485/9,0,Emission: 512/9,0
    Optics: Bottom,Gain: 75

  - Filter Set 3
    Excitation: 485/9,0,Emission: 512/9,0
    Optics: Bottom,Gain: 100
  Read Speed: Normal, Delay: 100 msec, Measurements/Data Point: 10

- Read RFP:
  Fluorescence Endpoint of full plate
  - Filter Set 1:
    Excitation: 555/9,0,Emission: 585/9,0
    Optics: Bottom,Gain: 100

  - Filter Set 2:
    Excitation: 555/9,0,Emission: 585/9,0
    Optics: Bottom,Gain: 150
  Read Speed: Normal, Delay: 100 msec, Measurements/Data Point: 10

Fluorescence analysis:

3 Grow desired bacteria overnight in a 50 ml falcon tub, containing **10 ml M9TG media** and grown cultures @ **37 °C** , 250 RPM overnight.

Luria-Bertani media is also possible to use, but cells must be washed with PBS buffer prior to plate reader experiment because of the high amount of fluorescence from the yeast extract:

After overnight incubation, spin cells down at 4700 x G, for 5 min, discard the supernatant and resuspend in 10 ml PBS.
Repeat prior steps and resuspension again in PBS.

4 Load **160 µl PBS buffer** in the wells of the 96-well plate (with clear bottom and black sides) and add **40 µl of overnight culture**

When cell have been grown overnight in LB media, washing steps are require before continuing! (see step 3)

Add as s control a sample with **160 µl PBS buffer** *and* **40 µl of non-fluorescent bacteria** .

5 Start plate reader protocol **go to step #2**

Analyses

6 Retrieve the data from the computer, correct for the measurements for the OD600 and subtract the autofluorescence from the control sample of all the other samples.