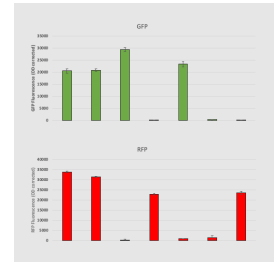


Oct 16, 2019

GFP-RFP plate reader assay

DOI

dx.doi.org/10.17504/protocols.io.784hryw



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Protocol Citation: Sebastiaan Kuiper 2019. GFP-RFP plate reader assay. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.784hryw>

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Protocol status: Working

We use this protocol and it's working

Created: October 16, 2019

Last Modified: October 16, 2019

Protocol Integer ID: 28668


Keywords: Green fluorescent protein, GFP, Red fluorescent protein, RFP, Plate reader, dCas, dCpf1, Inhibition, Escherichia coli, E. coli, fluorescence measurements of green fluorescent protein, green fluorescent protein, red fluorescent protein, fluorescence measurement, fluorescence, rfp plate reader, gfp, rfp, protein


Abstract

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

Materials

MATERIALS

 96-well plate, flat bottom, tissue culture treated, black wall with clear bottom **Fisher Scientific Catalog #3904**

 Microplate Reader Synergy Mx

 50 ml Falcon tube

Troubleshooting

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
	Na ₂ HPO ₄	1.44 g	10 mM
	KH ₂ PO ₄	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: 100Read 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: 150Read 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.

Note

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


Note

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

Note

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.

