Oct 16, 2019

OD and GFP Plate Reader Assay (72 h Measurement)

DOI
dx.doi.org/10.17504/protocols.io.8aihsce

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iGEM Wageningen 2019

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OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.8aihsce

Protocol Citation: Alba Balletbó 2019. OD and GFP Plate Reader Assay (72 h Measurement). **protocols.io** **https://dx.doi.org/10.17504/protocols.io.8aihsce**

Protocol status: Working
We use this protocol and it's working

Created: October 16, 2019

Last Modified: October 16, 2019

Protocol Integer ID: 28714

Abstract

Fluorescence measurements of Optical Density (OD) and Green fluorescent protein (GFP) in *Escherichia coli*.

Materials

MATERIALS

Microplate Reader Synergy Mx

Nunc™ MicroWell™ 96-Well Microplates, F 96 well plate, cell culture, clear, with lid, Sterile **Thermo Fisher** **Catalog #**167008

Media Preparation

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

M9TG Media (Without carbon source):

	Reagent	Amount to add for 50 mL
	M9 Salts	1X
	Tryptone	0.5 g

M9TG Media (0.5% Glycerol):

	Reagent	Amount to add for 50 mL
	M9 Salts	1X
	Tryptone	0.5 g
	Glycerol	0.25 g

M9TG Media (2% Glycerol):

	Reagent	Amount to add for 50 mL
	M9 Salts	1X
	Tryptone	0.5 g
	Glycerol	1 g

M9TG Media (0.5% Succinate and 1mM Leucine):

	Reagent	Amount to add for 50 mL
	M9 Salts	1X
	Tryptone	0.5 g
	Succinate	0.25 g
	Leucine	6.56 mg

Bacteria and Plate Reader Preparation

2 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.

3 Load 190 µL of the corresponding buffer in the wells of the 96-well plate and add 10 µL of overnight culture.

Note

The amount of buffer and overnight culture will depend on the OD after overnight bacterial cultivation. The amounts present in this protocol where determined by an OD ~0.3.

Note

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

Plate Reader Protocol

4 Set the following variables for the BioTek Synergy MX Microplate Reader:

- Temperature: Setpoint 37°C

Preheat before moving to next step. Start Kinetic [Run 72:00:00, Interval 0:04:20]

- Shake: Medium for 0:05 (MM:SS)

- **Read OD600**:
  Absorbance Endpoint of full plate
  Wavelengths: 600
  Read Speed: Normal

- **Read GFP**:
  Fluorescence Endpoint of full plate
  - Filter Set:
    Excitation: 488/9,0
    Emission: 510/9,0
    Optics: Bottom
    Gain: 100
    Read Speed: Normal

5 Start plate reader protocol.

Data Analysis

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.