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OD and GFP Plate Reader Assay (72 h Measurement)

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Protocol status: Working

We use this protocol and it's working



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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**

Troubleshooting

Media Preparation

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

M9TG Media (Without carbon source):

	Rea gent	Am mou nt to add for 50 mL
	M9 Salts	1X
	Tryp tone	0.5 g

M9TG Media (0.5% Glycerol):

	Rea gent	Am mou nt to add for 50 mL
	M9 Salts	1X
	Tryp tone	0.5 g
	Glyc erol	0.25 g

M9TG Media (2% Glycerol):



	Rea gent	Am mou nt to add for 50 mL
	M9 Salts	1X
	Tryp tone	0.5 g

	Glycerol	1 g
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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 were determined by an OD ~0.3.

Note

When cells have been grown overnight in LB media, washing steps are required 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.