Platereader workflow with V. natriegens

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ABSTRACT

This protocol provides a workflow for platereader measuremtns with V. natriegens.

TAGS

plate reader

GFP

Show tags

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

MATERIALS TEXT

transparent and black 96-well plate

Sample preparation

1 Aliquot 50 µL LBv2 in 1.5 mL reaction tubes

2 Transfer material from glycerol stock into these reaction tubes

3 Prepare transparent 96 well plat with 190 µL LBv2

4 Use 10 µL of LBv2 with cells from glycerol stock to inoculate the 96 well plate. (Carry out experiment with four technical replicates

Preculture

5 Incubate 96 well plate in a platereader (Protocol: Preculture)

Preculture:
37 cycles
600 s cycle time
Shaking: Double orbital, 500 rpm
Protocols: OD + optional protocols

OD600 (Absorbance):
Wavelength: 600 nm
Settling Time: 0.5 s
No. of flashes: 30

6 Dilute grown cultures 1:40 (195 µL LBv2 + 5 µL culture) in a sterile 96 well plate

7 Apply Shaking protocol

Shaking (Absorbance):
Shaking: Double Orbital, 300s, 700 rpm
Measure OD600

8 Dilute grown cultures 1:50 (196 µL LBv2 + 4 µL culture) in a black sterile 96 well plate

Measurement

9 Start protocol for measurement in the plate reader (Protocol: OD+Lux, OD+GFP, OD+RFP)

OD + Lux
121 cycles
300 s cycle time
Shaking: Double orbital, 500 rpm
Protocols: OD + luminescence

OD600 (Absorbance):
Wavelength: 600 nm
Settling Time: 0.5 s
No. of flashes: 30

Luminescence:
Gain: 4000
Measurement interval time: 1 s