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Platereader workflow with V. natriegens

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

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Abstract

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

Materials

transparent and black 96-well plate

Troubleshooting

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