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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 measurements with *V. natriegens*.

Materials

transparent and black 96-well plate



Sample preparation

- 1 Aliquot 50 μ L 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 μ L LBv2
- 4 Use 10 μ L 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 μ L LBv2 + 5 μ L 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 μ L LBv2 + 4 μ L 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