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Growth_of_Pseudomonas_fluorescence_SBW25_in_48-well_Plate_Format

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Protocol status: Working

We use this protocol and it's working

Created: September 22, 2022



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Protocol Integer ID: 70401

Keywords: pseudomonas fluorescens sbw25, culturing pseudomonas fluorescens sbw25, epoch2 plate reader, suitable for many pseudomonas strain, many pseudomonas strain, other plate reader, plate reader, biotek epoch, well microplate, od reading, m9 minimal media recipe, plate lid, nrel team for the m9 minimal media recipe

Abstract

This protocol provides a method for culturing *Pseudomonas fluorescens* SBW25 in 48-well microplates. The protocol is specific for use with a BioTek Epoch 2 plate reader, but other plate readers can be used with appropriate modifications. One advantage of the Epoch 2 instrument is the ability to run with the plate lid at a higher temperature than the well contents. This prevents condensation on the lid and resulting noise in the OD readings.

This protocol is suitable for many *Pseudomonas* strains.

I thank members of the ORNL team, Joshua Elmore and Quint Peabody V, for assistance during development of this protocol and tips on use of the Epoch2 plate reader. I thank the NREL team for the M9 Minimal Media recipe.



Materials

48-well cell culture microplates (Greiner Bio-One 677180)

Note: If using an Epoch2 microplate reader, the physical maximum plate height is 23.5 mm.
The Greiner plates (with lids) have a maximum height of 22 mm.

50 mL Bio-Reaction tubes (CELLTREAT 229475)

LB medium

- 10 g Tryptone
- 5 g Yeast Extract
- 10 g NaCl

Add 1 L deionized water

Sterilize by autoclaving at 15 psi, 121-124°C for 20 minutes

M9 Minimal Medium (1L):

1. To 800 mL H₂O, add the following in the order below, mixing in between:

- 0.1 mL 1M CaCl₂
- 0.18 mL 100 mM FeSO₄*
- 2.0 mL 1M MgSO₄
- 100 mL 10X M9 minimal salts

2. Add carbon source, and add H₂O to bring the final volume up to 1 L.

3. Filter sterilize.

*Optional. Reduces fluorescent siderophore secretion in *Pseudomonas* strains. A 100 mM stock of FeSO₄ should be aliquoted quickly and frozen to prevent oxidation.

10X* M9 Minimal Salts (1L):

<u>Ingredients:</u>	<u>(g/L)</u>
Disodium phosphate (anhydrous)	67.8
Monopotassium phosphate	30
Sodium chloride	5
Ammonium chloride	10

* A 5X stock could be prepared instead



1. Dissolve the above (or 112.8 g M9 Minimal Salts, 5X, Sigma M6030) in 1L of distilled water.
2. Autoclave in liquid cycle or filter sterilize.



Culturing *Pseudomonas fluorescens* SBW25

1 DAY1: Overnight culture

Inoculate  5 mL LB, in a vented or loosely capped culture tube (e.g. CELLTREAT 50 mL Bio-Reaction tube), from a glycerol stock or with a single colony from a plate.

Grow overnight at  30 °C with shaking at  250 rpm .

DAY2: Four hour culture

NOTE: *Pseudomonas* cells typically have a long lag time if transferred from a stationary-phase overnight culture directly to minimal medium. The additional four hour culture period in fresh LB allows the cells to return to exponential growth phase, and minimizes the subsequent lag upon transfer to minimal medium.

Dispense  10 mL LB into vented or loosely capped 50 mL culture tube.

Inoculate with  250 µL overnight culture.

Incubate for four hours at  30 °C with shaking at  250 rpm

Pellet cells by centrifugation,  4000 rpm for  00:05:00 .

Pour off supernatant and resuspend in  5 mL M9 salts.

Collect cells by centrifugation, and repeat M9 salts wash step.

Resuspend pellet in  5 mL sterile M9 salts.

Remove a small aliquot to measure OD600 on a spectrophotometer.

Plate set-up and data collection

- 2 Prepare M9 minimal medium containing desired carbon source (M9-C) and filter sterilize. Dilute washed cells to a final OD600 of 0.05 to 0.1 OD with M9-C. Dispense 600 µl/well to 48-well plate and cover with the supplied lid. Each unique well condition or sample type should be performed in triplicate.

NOTE: If long incubation periods are anticipated (>24 hours), fill edge wells with M9-C and only use the 24 inner wells for culturing. This will minimize evaporation during long culture times.

Start up the Gen5 software on the Epoch2 system and place the plate in the drawer.

Enter the protocol shown below; run times and sampling intervals can be adjusted as needed.

48-well plate notes:

Notice that we have left the "Use Lid" box unchecked (even though we use the lid). This is because the Default 48 well plate plus lid is higher than the instrument max of 23.5 mm. To prevent error message, leave lid box unchecked or generate a custom plate description for the Greiner plate (System>Plate Types>View/Modify).

Procedure - Epoch 2 (Com1)

Select steps

Actions

Read

Set Temperature

Shake

Kinetic

Start Kinetic

Monitor Well

Append Reads

Pause

Delay

Plate Out/In

Stop/Resume

Other

Comment

Options

Plate Type: 48 WELL PLATE ☐ Use lid

☐ Cuvette

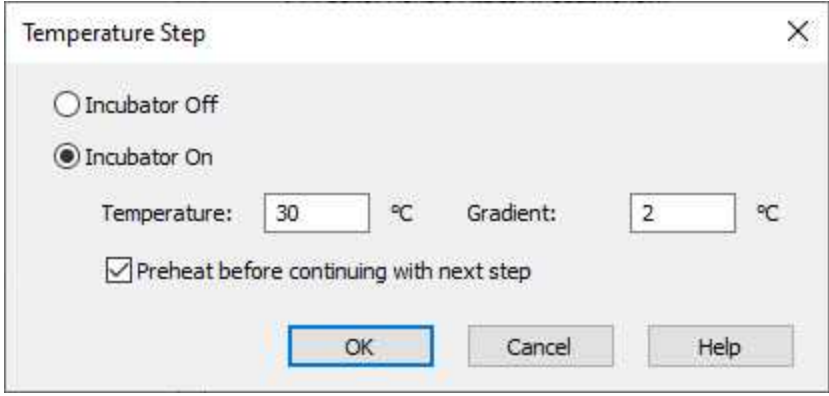
Select wells: ☒ Per step ☐ At runtime

Description	Comments
Temperature: Setpoint 30 °C	
Start Kinetic [Run 96:00:00, Interval 0:15:00]	
Shake: Double Orbital (Continuously)	
Read: (A) 600	
End Kinetic	

Validate OK Cancel Help

Adjusting the Well and Lid temperature:

The Epoch 2 allows the lid to be run at a higher temperature to avoid condensation. This is set in the Gradient box below. This should be set to 1 or 2°C.



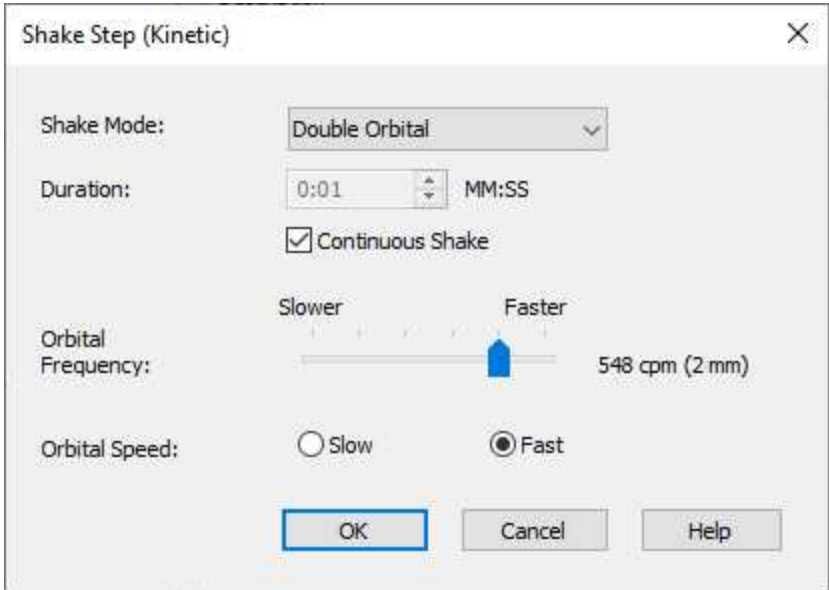
Temperature Step [X]

☐ Incubator Off
☒ Incubator On

Temperature: °C Gradient: °C

☒ Preheat before continuing with next step

Adjusting the Shaking mode and speed:



Shake Step (Kinetic) [X]

Shake Mode: ▾

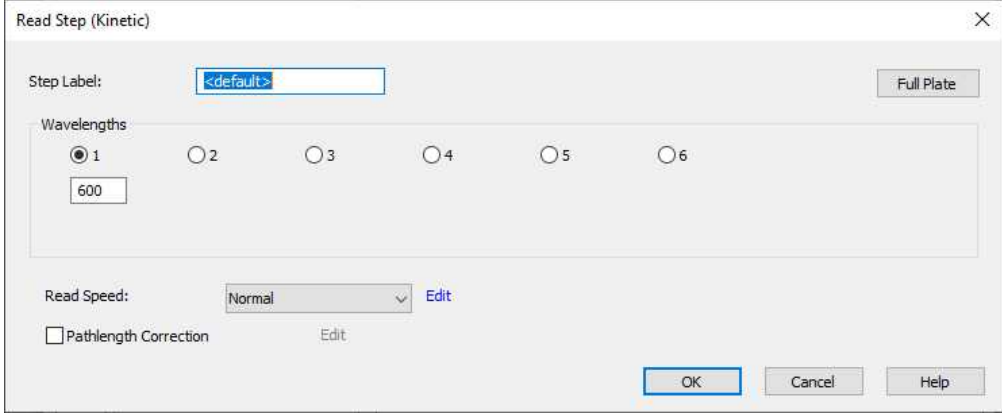
Duration: MM:SS
☒ Continuous Shake

Orbital Frequency: Slower Faster
 548 cpm (2 mm)

Orbital Speed: ☐ Slow ☒ Fast

Adjusting the Read step:

Edit the Read Speed to bring up the second window. Increase the delay time to 250 msec to allow wells to stabilize before reading.



Read Step (Kinetic)

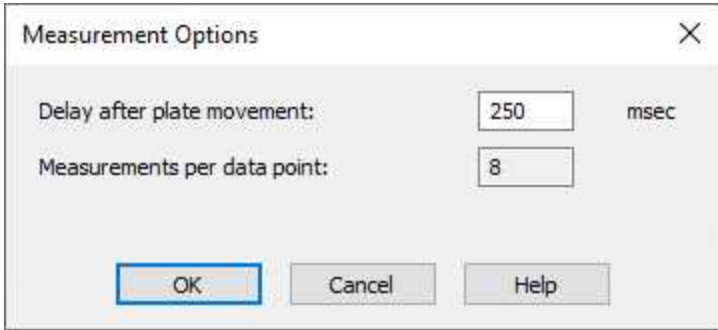
Step Label:

Wavelengths

☒ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6

Read Speed:

☐ Pathlength Correction



Measurement Options

Delay after plate movement: msec

Measurements per data point:

Once protocol has been set up, start the run and monitor data collection over time. The run can be stopped at earlier timepoints without losing the data.