Oct 07, 2022

Conditions for growth of Rhodobacter sphaeroides in different media

Jaya K Yakha[1], Deborah K Hanson[1], Rosemarie Wilton[1], laible[1]

[1]Argonne National Lab

1 Works for me Share

dx.doi.org/10.17504/protocols.io.kxygx9emog8j/v1

Jaya K Yakha

ABSTRACT

This protocol provides a method for culturing *Rhodobacter sphaeroides* wildtype 2.4.1 and its KO strains in shake flask and BioTek Epoch 2, 48-well microplates. A baffled side arm flask is used but other flasks can be used with appropriate modifications.

DOI

dx.doi.org/10.17504/protocols.io.kxygx9emog8j/v1

PROTOCOL CITATION

Jaya K Yakha, Deborah K Hanson, Rosemarie Wilton, laible 2022. Conditions for growth of Rhodobacter sphaeroides in different media . **protocols.io** https://dx.doi.org/10.17504/protocols.io.kxygx9emog8j/v1

LICENSE

This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

CREATED

Sep 20, 2022

LAST MODIFIED

Oct 07, 2022

PROTOCOL INTEGER ID

70308

Citation: Jaya K Yakha, Deborah K Hanson, Rosemarie Wilton, laible Conditions for growth of Rhodobacter sphaeroides in different mediaÃÂ https://dx.doi.org/10.17504/protocols.io.kxygx9emog8j/v1

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium,

MATERIALS TEXT

- Incubator at 33^{0}C
- ^{G}YCC agar plate and liquid medium
- Centrifuge
- Sterile tooth pick
- Colorimeter/spectrophotometer

Conditions for growth of *Rhodobacter sphaeroides* in GYCC medium in shake flasks

1 Inoculate a ^{G}YCC agar plate from a glycerol stock containing appropriate antibiotic (if necessary) and incubate at **33 °C** in an incubator in dark.

2 After **72:00:00** pick one colony from the plate and inoculate 80 ml fresh ^{G}YCC medium ^{3d} with appropriate antibiotic (if necessary) in **125 mL** baffled side arm flask.

3 Incubate at **33 °C** shaking at **125 rpm** for 72 -96 hours.

4 Monitor the growth of the culture with regular measurement of turbidity. Side-arm flasks are especially convenient for the determination of culture turbidity with a Klett-Summerson colorimeter. The equivalent OD_{600} may also be used if side arm flasks are not used.

Conditions for growth of *R. sphaeroides* in ^{G}YCC or MR26 medium in Microplate

5 Inoculate ^{G}YCC or MR26 agar plates from a glycerol stock containing appropriate antibiotic if necessary and incubate at **33 °C** .

6 After **72:00:00** s pick one colony from the plate and inoculate 25 ml fresh medium of ^{G}YCC ^{3d} or MR26 containing appropriate antibiotic (if necessary) in **50 mL** flask

7 Incubate at **33 °C** with shaking at **125 rpm** for 72 -96 hours.

Citation: Jaya K Yakha, Deborah K Hanson, Rosemarie Wilton, laible Conditions for growth of Rhodobacter sphaeroides in different mediaÃÂ https://dx.doi.org/10.17504/protocols.io.kxygx9emog8j/v1

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium,

8 Monitor the growth of the culture with measurement of turbidity. Once the culture reaches to the log phase (OD_{600}~0.7), the starter culture is ready for inoculation of the microplate.

9 For each well 600, µl of fresh ^{G}YCC or MR26 medium was added. It is recommended to use Microplate Greiner BioOne 48 well (Item no. 677180) for compatibility with a BioTek Epoch2 microplate reader

Procedure Details in microplate reader

9.1 Plate Type: Greiner BioOne 48-wellv2 (Use plate lid)
Eject plate on completion
Set Temperature: Setpoint 33°C, Gradient 2 °C
Start Kinetic: Runtime 96:00:00 (HH:MM:SS), Interval 0:15:00, 385 Reads
Shake Double Orbital: Continuous
Frequency: 548 cpm (2 mm)
Read:A600
Absorbance Endpoint
Full Plate
Wavelengths:600
Read Speed: Normal, Delay: 200 msec, Measurements/Data Point: 8
End Kinetic

10 Once the run is complete, export the data in a .txt or .xls file and save in an appropriate location. Analyze and visualize the data by any software.

Citation: Jaya K Yakha, Deborah K Hanson, Rosemarie Wilton, laible Conditions for growth of Rhodobacter sphaeroides in different mediaÃÂ https://dx.doi.org/10.17504/protocols.io.kxygx9emog8j/v1

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium,