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Growth curves

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[1]In-house protocol

1 Works for me This protocol is published without a DOI.

Eadewunm

ABSTRACT

Rules of thumb and directions for making growth curves

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GUIDELINES

- Rules of thumbs
- Making an overnight culture
- In the morning
- Growth curve

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ABSTRACT

Rules of thumb and directions for making growth curves

Rules of thumb

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1 Never remove > 1/3 of a culture for a growth curve

2 Read OD_{600} (optical density at 600 nm) of 200 microliters of culture added to 800 microliters of fresh media → 1,000 microliters of fluid in cuvette; = 1:5 dilution
- Remember to set the factor equal to 5 when reading from the spec

3 Easier to measure 800 microliters of fresh media into cuvettes ahead of time; simply store in cuvette box

4 Easier to measure media into a 50 mL conical for filling fresh cuvettes; less likely to get a contaminated bottle

5 Easier to use same blank multiple times; suggest every time you refill conical with media for cuvettes, make a new blank with that and use with cuvettes from that mixture.

6 Place cuvettes into 10% bleach solution when finished *This is a must for BSL 2 organisms such as *E. faecalis*.

Making an overnight culture

7 Measure 5mL appropriate media with appropriate antibiotics into fresh conical (make sure to label conical with date, strain!)

8 Take 1 colony from a plate with appropriate strain (use pipette to poke that colony)

9 Inoculate media

10 Grow overnight (usually start culture ~3-5 p.m. night before culture is needed)

In the morning:

11 Measure OD_{600}

12 Use $V_1C_1=V_2C_2$ to make a 0.01 OD_{600} dilution in media for growth curve

13 Place culture in flasks, mix, place in appropriate incubator, start count up timer.

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Growth curve:

14 The first time point is at 30 min on count up timer.

15 Take time point every 30 min, write down data and graph as you go

16 Use the count-up timer to measure time. When it reaches 30 min, 1 hour, 1 ½ hours, etc. remove cultures from incubator, pipette into cuvettes (remember 1/5 dilution, e.g. 200 microliters culture in 800 microliters media)

17 Remember to mix culture gently before adding to cuvette (the culture can be clumpy at the bottom, and you want a homogenous solution)

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