Nov 23, 2020

Toxicity Assay

Elizabeth Fozo[1]
[1]In-house protocol

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

Eadewunm

ABSTRACT

Toxicity Assay (based on growth curves protocol)

PROTOCOL CITATION

Elizabeth Fozo 2020. Toxicity Assay. **protocols.io** https://protocols.io/view/toxicity-assay-bpwhmpb6

CREATED

Nov 19, 2020

LAST MODIFIED

Nov 23, 2020

PROTOCOL INTEGER ID

44713

ABSTRACT

Toxicity Assay (based on growth curves protocol)

Rules of thumb

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

2 Read OD600 (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 spectrometer

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 and EHEC.

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:

In the morning

11 Measure OD600

12 Use V1C1=V2C2 to make a 0.01 OD600 dilution in media for growth curve

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

Toxicity Assay:

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

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

16 Use count up timer to measure time. When it reaches 30 min, 1 hour, 1.5 hours, etc. remove cultures from the 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 a cuvette (the culture can be clumpy at the bottom, and you want a homogenous solution)

18 Once the culture reaches OD 0.3 (at about 2-3 hours for E. coli in LB), split cell culture in half.

Try to reach as close to OD 0.3 as possible. May require you to take samples off the 30-minute cycle.

19 Induce ½ cells with the appropriate inducer (for example, arabinose) at the appropriate final concentration (for ara, 0.2% is saturating).

20 Continue incubation for all cultures, taking OD600 every 30 minutes after induction.