Determining biofilm growth amount (absorbance) V.2

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DISCLAIMER

ABSTRACT

This protocol describes a method to determine the growth amount of biofilm at the early stage of biofilm formation by measuring absorbance. Here we use our own engineered bacteria, and it requires induction of IPTG and cultured with silver ion. You may use a different kind of bacteria and different culturing medium.

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MATERIALS TEXT

(Optional) IPTG, silver nitrate, *Escherichia coli*, LB broth medium.

Droppers, forceps, flasks with caps, 50 mL centrifuge tubes, MBBR carrier K1, oven, 1X phosphate-buffered saline (PBS), centrifuge, spectrometer.

DISCLAIMER

IPTG induction

1 *Escherichia coli* grown overnight was diluted by LB to OD600=0.4-0.6.

2 IPTG was added to cell culture to 1mM IPTG finally, and incubated 3h at 171 rpm, 37°C in orbital shaking incubator.

Sample preparation

3 Preparing several 100mL flasks by filling the flasks with MBBR carrier K1. Autoclave all the flasks and dry them in an oven at 60s°C.

Note

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4 Cells cultures and silver nitrate (to 6µM) solution were mixed in advance. Add the mixture into each bottle.

Note

5 Incubate all bottles in a biochemical incubator at 37 °C .

10m

Absorbance measurement

6 At the day of measuring the absorbance, take the flasks to be tested out from incubator.

7 Using a pair of forceps, carefully pick out the carriers that has not been submerged into the culture and discard.

Note

8 Randomly pick five carriers out from culturing flask and put into a 50 mL centrifuge tube. Label this tube as "Washing tube".

Note

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9 Wash these five carriers using 1X phosphate-buffered saline (PBS) for three times. Collect all eluents.

Note

10 Use a dropper, add 2-3 mL 1X PBS into the Washing tube. Cap the tube and rinse every corner of the inside wall of tube using the PBS added by rotating the tube. Collecting the eluents by pouring these PBS into Eluent tube. Repeat these steps for three to five times.

Note

11 Centrifuge the Eluent tube at 10000 rpm, Room temperature, 00:10:00 . 10m

12 Discard all supernatants.

Note

13 Add 3 µL 1X PBS into Eluent tube. Resuspend the pellet.

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14 Measure the absorbance of the liquid 13 at OD600. Record the absorbance.

15 Repeat steps from Step 7 to Step 14 for three times. Calculate the mean value for the three measurements. A growing curve of biofilm can be generated from the data collected.

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