Method Sheet 02

Preparing a bacterial culture for screening in 96-well format

Overview

This method sheet explains how to prepare a bacterial culture of *Escherichia coli* DH5α or *Micrococcus luteus* before screening for inhibitors of bacterial growth in 96-well format.

Equipment

- Shaking incubator capable of incubating at 37°C
- Fixed incubator capable of incubating at 37°C
- Fridge to store agar plates at 4°C
- *Optional*: A class I or class II biosafety cabinet will be helpful to aliquot reagents

Reagents

- An agar plate with live colonies of *Escherichia coli* DH5α or *Micrococcus luteus*, or another microbe of your choice
- A 500 ml bottle of sterile Luria broth (LB)

Method

1) The day before preparing the plate, aliquot 5 ml of LB (without antibiotic supplements) into a 25 ml sterile tube.
2) Inoculate the LB media with bacteria using a sterile loop or plastic pipette tip by touching a single colony from the agar plate containing your microbe of interest and then swirling the end of the loop or tip briefly in the medium.
3) Wrap the agar plate in film and return to storage at 4°C for up to two weeks.
4) Replace the lid on the tube, but leave slightly loose to allow air to enter.
5) Incubate the tube in a shaking incubator overnight at 37°C.

~ Overnight incubation step ~

6) Retrieve the tube from the shaking incubator, which should now contain a bacterial culture at stationary phase (see notes below).
7) Pipette 11 µl of the overnight culture into 11 ml of fresh LB in a sterile tube without antibiotic and mix by gentle inversion (this dilution is a commonly used shortcut for assay preparation, but if prefered, a more consistent approach to seeding at a specific cell density is given in the notes section below).
8) If you are making more than one 96-well plate, simply multiply the volumes shown in step 7 by the number of plates you intend to set up.
9) Using sterile technique, use a multichannel pipette to aliquot 100 µl of this suspension into a sterile 96-well plate (follow Method Sheet 03).

10) Once all plates have been seeded with bacterial cultures, they are ready to challenge with natural compounds or extracts for screening (follow Method Sheet 04).

Notes

- Always wear appropriate personal protective equipment (lab coat, gloves, safety glasses) and follow your local laboratory good practice rules when working with these or any other micro-organisms.
- Even though *Escherichia coli* DH5α and *Micrococcus luteus* are considered to be in Hazard Group 1, which means they are organisms that have very low potential to cause harm to those working with them, you should follow strict biosafety protocols.
- These organisms can be cultured in any Containment Level 1 lab space with a suitable waste stream (i.e. autoclaving of solid waste, disinfection of liquid waste).
- If you choose to study a different organism, check which Hazard Group it belongs to, and ensure you work only in a lab space operating at a Containment Level suitable for that organism.
- For example, if your organism is in Hazard Group 2, culture only in Containment Level 2 using appropriate biosafety protocols.
- A stationary phase culture of *E. coli* DH5α typically has an optical density (OD) at 600 nm of around 2.0, and a cell density of ~2 x 10^9 CFU/ml.
- Diluting the culture 1,000-fold in this protocol yields a suspension of ~2 x 10^6 CFU/ml, day to day variations in this concentration are corrected for by normalisation to the control condition during the data analysis phase.
- However, if you would like to prepare plates a more consistent seeding concentration, it is possible to measure the absorbance of the overnight culture at 600 nm and prepare dilutions accordingly to yield the same estimated CFU/ml each time.
- *Micrococcus luteus* grows more slowly and to a lower density than *E. coli* in LB, reaching OD at 600 nm of ~1.0 and ~5 x 10^8 CFU/ml by 24 hours, but good assay screening results can still be obtained using the same dilution protocol given above (i.e. 1:1,000).

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