Method Sheet 29

Investigating the effects of hit extracts on kinetics of bacterial growth

Overview

This method sheet explains how to investigate the impact of hit extracts at a concentration of 100 µg/ml on the kinetics of bacterial growth using the microplate absorbance method.

Reagents

- One or more hit natural extracts at 10 mg/ml in DMSO

Equipment

- Multichannel pipette (8- or 12-channel) capable of dispensing 50 - 200 µl
- Sterile pipette tips (compatible with each pipette)
- Static (non-shaking) 37°C incubator
- Waste container for used tips

Method

1) Fully defrost one or more of the hit natural extracts.
2) Prepare a 96-well plate containing bacterial cell cultures at 90 µl per well (follow Method Sheet 03, this should be done early in the morning on the same day as challenge).
3) Prepare dilutions of each hit extract by combining 5 µl of hit extract with 45 µl of sterile LB medium in a sterile microtube and mixing well.
4) Prepare a negative control microtube containing 45 µl of LB and 5 µl of DMSO, and a positive control microtube with 50 µl of LB containing 100 µg/ml ampicillin (or another suitable antibiotic).
5) Supplement 4 wells of the plate with 10 µl of control suspension (LB only) per well, according to the plate map shown below:

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		Neg control	Extract 1	Extract 2	Extract 3	Pos control						
C		Neg control	Extract 1	Extract 2	Extract 3	Pos control						
D		Neg control	Extract 1	Extract 2	Extract 3	Pos control						
E		Neg control	Extract 1	Extract 2	Extract 3	Pos control						
F												
G												
H												

6) For each of the different hit extract suspensions, supplement a separate set of 4 wells of the plate with 10 µl of each hit extract suspension (three extracts are tested in this example, but feel free to adjust to the number of extracts you are testing).
7) Supplement a different set of 4 wells with 10 µl of positive control suspension per well
8) Note that we are avoiding use of the outer wells of the plate to minimise errors arising from the 'edge effect'.
9) The other wells on the plate receive no supplement (i.e. they remain only 90 µl culture).
10) Move the culture plate backwards and forwards, then side to side, gently several times to mix the suspension into each culture.
11) Avoid jarring movements that may splash liquid between wells and cause cross-contamination.
12) Measure the absorbance of the plate straight after challenge (to obtain the baseline, time = 0 h readings) using a microplate reader (follow Method Sheet 05)..
13) Place the culture plate in a static 37°C incubator to allow growth to begin (does not need to be a shaking incubator).
14) Remove the plate at hourly intervals, and read the absorbance again, once per hour, using the same wavelength settings as for the first (baseline) read.
15) Return the plate to the 37°C incubator in between each measurement to allow the cultures to continue to grow.
16) Repeat for up to 8 measurements, once per hour, on day 1.
17) Measure the growth once more on day 2, at 24 hours after the first measurement (i.e. at the same time as the first measurement was taken the day before).
18) The plate can now be discarded.
19) Repeat this experiment a further three times in separate plates to obtain four experimental replicates.
20) Proceed to analyse the growth curve data as shown in Method Sheet 30.

Notes

- As the stock extracts are at 10 mg/ml, and the first dilution is 1:10 into LB to yield a 1 mg/ml substock, the final supplementation of 10 µl suspension onto 90 µl culture is another 1:10 dilution to yield a final concentration of extract in each well of 100 µg/ml.
- In this example, each plate contains four technical replicates, which is not the same as four experimental replicates.
- The same experiment should be set up 4 times on separate plates to achieve the necessary four independent experimental replicates.
- You can attempt to prepare the extracts yourself, or alternatively resupplies of individual extracts are available economically and promptly from Caithness Biotechnologies.