Antimicrobial activity assay

Bie Ekblad

Abstract

Citation: Bie Ekblad Antimicrobial activity assay. **protocols.io**
dx.doi.org/10.17504/protocols.io.i66chhe
Published: 21 Aug 2017

Guidelines

Be careful not to contaminate neighbouring wells/rows with the peptide added. If the peptides are highly potent it is a good idea to separate different peptides added (or at different starting-concentrations) by an empty row in-between.

Before start

Prepare appropriate stock-solutions of peptide according to the concentration added in the first well of the microtiter plate.

Protocol

Overnight culture

Step 1.

Add indicator strain to e.g. 1 ml culture (depends on the amount you need next day) and grow overnight (o/n) at the desired temperature, with or without agitation according to indicator strain used.

Calculation step

Step 2.

Calculate the volume of peptide to add according to the concentration you need in the first well of the second row of the microtiterplate assay in 200 µl.

Example: You want a final concentration of 50 nM of peptide in the first well and the stock-concentration is 50 µM

This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

1. This means that you will need a peptide concentration of 0.2 µM in 50 µl solution of growth medium (before dilution):
   0.05 µM * 200 µl = x µM * 50 µl
   x = 0.2 µM
2. The peptide is diluted by a factor of 2 throughout each row of the microtiterplate and the first well of each row is thus added another 50 µl of medium that will be transferred to the next well of the same row after addition of peptide
3. You thus need to take into account this extra 50 µl of solution (I want a peptide concentration of 0.2 µM in 100 µl solution in order to have 0.2 µM in 50 µl solution after dilution):
   0.2 µM * 100 µl = x µl * 50 µM
   x = 0.4 µl
   The volume of peptide that needs to be added in the first well of the second row of the microtiterplate is thus 0.4 µl in order to achieve a concentration of 0.05 µM in 200 µl (in the first well).
   OR you can just calculate the volume you need in 200 µl and multiply by two...

Calculation step

Step 3.

Thaw peptides of choice on ice

Antimicrobial activity assay

Step 4.

Add 50 µl growth medium in all wells of each row you will be using.

NB! The first row (wells 1A-12A) is for medium and indicator cells only.

AMOUNT

50 µl Additional info: growth medium

Antimicrobial activity assay

Step 5.

Add the calculated amount of peptide (step 2) in the first well of each row (depending on how many rows and concentrations of peptide etc.)

Mix by pipetting gently up and down

Antimicrobial activity assay

Step 6.

Add growth medium to each well of the first column so that the final volume is 100 µl.

Mix gently by pipetting up and down 6-8 times

Antimicrobial activity assay

Step 7.

This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Transfer 50 µl from the first well to the second well in the same row and mix by pipetting up and down 6-8 times.

Do this for each well until you reach the final well of this row.

After mixing, throw away the final 50 µl of solution so that each well now has a final volume of 50 µl (containing peptide and growth medium with two-fold dilutions).

Antimicrobial activity assay

Step 8.

Dilute the o/n-culture e.g. 1:50 with fresh growth medium for incubation during day-time or e.g. 1:200 for incubation o/n.

Antimicrobial activity assay

Step 9.

Add 150 µl of diluted o/n-culture to each well of each row so that each well now has a final volume of 200 µl (start to add culture in the most diluted sample, i.e. well 12).

AMOUNT

150 µl Additional info: diluted o/n culture

Antimicrobial activity assay

Step 10.

Put the lid on and incubate the microtiter plate assays at your temperature and time of choice.

Determining MIC50

Step 11.

After incubation, remove the solution in well 1A, wash and add 200 µl of fresh growth medium from the same batch (well 1A now serves as the background-control). Measure the OD spectrophotometrically at 600 nm in a mirotiterplate reader (make sure to add 'shaking-time' in the program so that the cells are well dispersed before measuring).

Determining MIC50

Step 12.

1. Subtract the value from well 1A.
2. Calculate the average growth of indicator cells without peptide added (from the first row).
3. Divide this average growth-value by two - this is 1/2 max of growth, e.g. 0.15
4. Locate the 1/2 max at the row where peptide is added which is normally in the middle of two wells, e.g. wells 5B and 6B
5. Note the value of OD for these two wells, e.g. 5B = 0.112 and 6B = 0.215

This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

6. Note the concentration of peptide added in these two wells, e.g. 5B = 3.125 nM and 6B = 1.563 nM
7. calculate the ΔC (difference in concentration, e.g. 1.563 nM) and the Δ OD (e.g. 0.103)
8. The fraction-OD: Subtract 1/2 max value from OD-value of the first of these two wells (e.g. well 5B) and divide by Δ OD, e.g. (1/2 max/0.112)*0.103
9. The MIC-value is the concentration of the first of these two wells (e.g. 5B) minus (the fraction-OD multiplied by ΔC), e.g. 3.125 nM - (0.103*1.563 nM) = 2.95 nM ≈ 3 nM

The MIC_{50} is presented as the average value of at least three independent measurements (at different time points).

This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited