

Minimal Inhibitory Concentration (MIC)

Preparation:

- Add 21g of Mueller Hinton broth powder in 1 L of distilled water, and sterilize by autoclaving at 121°C for 15 minutes.
- The McFarland standard is used to adjust the turbidity of bacterial suspensions. The 0.5 McFarland standard is made by mixing 99.5 ml 0.18 M H₂SO₄ and 0.05 ml 0.048 M BaCl₂ to form barium sulfate precipitate. The absorbance of 0.5 McFarland standard at 625 nm is approximately 0.13, which determines the approximate cell density is 10⁸ CFU/ml.

Test procedure:

1. Incubate 5 ml Mueller Hinton Broth with bacteria at 37 °C overnight.
2. 50 µl bacterial solution was taken out and diluted 100 times with 5 ml new medium, then continued to incubate at 37 °C for 2 hrs.
3. Detect the absorbance of the bacterial solution at 625 nm and dilute the original solution to OD₆₂₅ value is around 0.13
4. Dilute the standard bacterial solution to 10⁶ cell/ml
5. Take a 96-well plate and transfer 50 µl medium from column 2 to column 12
6. Transfer 100 µl antimicrobial peptide solution in column 1
7. Add 50 µl ampicillin in column 12 as the negative control
8. Serial two-fold dilution of peptide solution was taken from column 1 to column 10.
9. Add 50 µl bacterial solution from column 1 to column 11, and the final bacterial concentration is 5*10⁵ CFU/ml.
10. Place the plate in the incubator at 35 °C overnight.
11. Detects the absorbance of each well at 625 nm.

Measure MIC: Fitting of a modified Gompertz function^[1]

Data in the form of log concentration(x) and fractional area(y) can be analysed by using a altered Gompertz equation.

$$y = A + Ce^{-e^{B(x-M)}}$$

A is the lower asymptote of y, B is a slope parameter, C is the distance between the upper and lower asymptote, and M is the log concentration of the inflexion point. The value of MIC is defined as the intersection of the lines y = A with the equation of the line tangential to the point (M, (A + C^{-e}))

$$\text{MIC} = 10^{\left(M + \frac{1}{B}\right)}$$

Reference:

1. Lambert, R. J., & Pearson, J. (2000). Susceptibility testing: accurate and reproducible minimum inhibitory concentration (MIC) and non-inhibitory concentration (NIC) values. *Journal of applied microbiology*, 88(5), 784–790. <https://doi.org/10.1046/j.1365-2672.2000.01017.x>