

Performing A Dose-Response Curve

The dose response curve, also known as an antibiotic kill-curve, is a valuable tool to determine cell toxicity when exposed to various concentrations of antibiotic. Each time a new antibiotic is used, a new curve should be performed to ensure the proper dosage, while maintaining cell viability. This is one method to determine the best antibiotic concentration for the assay.

Cell Preparation

Determine the appropriate seeding volume for the cell line to be used. If using a 96-well plate, a concentration curve may be needed to determine the best volume of cells/well to allow for growth and the prevent death from over-crowding before application of antibiotic.

Note the time when cells reach their greatest confluence. This is when cells expand to optimally cover all area of the well before death and flotation occur as a result of entering the stationary phase and/or over growth. Cells must still be in log phase to allow for uptake of the antibiotic and resulting response.

Antibiotic Preparation

Dilute the antibiotic to a broad linear concentration range, using the appropriate growth medium as the diluent. The wider the concentration range, the better the assessment of optimal user concentration.

The following is a recommended procedure for performing an antibiotic dose-response curve.

Procedure

1. Set cells in the pre-determined concentration and allow them to grow to confluence under normal growth conditions (temperature, CO₂, etc.).
2. Once confluent, dilute the antibiotic to concentrations for testing.
3. Remove the growth media from the cells, maintaining aseptic conditions.
4. Label the plate(s) to reflect the concentrations of antibiotic applied to the cells. The best maximum volume per well of 96-well plates may be 200 µL/well.
5. Apply the antibiotic to the respective wells, leaving one set of rows empty. To these wells, add growth medium not containing the antibiotic. These wells can be used to compare the cells under antibiotic influence and determine the maximum effectiveness and signal the end of the test. Return the plate to the proper growth conditions and observe daily, noting results.
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7. Optimal effectiveness should be reached in 2-10 days, depending on the antibiotic. This can be seen by almost total obliteration of cells in highest concentrations, and little to no effect in the lower concentrations.
8. Once the maximum effect has been reached, the cells can be stained using methylene blue and the optical density can be measured.
9. If the concentration range used in the study is broad enough, the optical densities can be graphed.
10. Several curves may need to be performed to determine the concentration that delivers the best effect of the cells without causing adverse reactions to the cells, themselves.
11. From the data obtained in the kill-curve, extrapolate the appropriate concentration of antibiotic to use to obtain the desired effect.
12. This kill-curve (dose-response curve) should be performed for each new lot of antibiotic, as each lot may vary in purity or potency. Cell conditions may also change.