

Alamar Blue Assay: Drug Sensitivity Test

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Abstract

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Protocol

Step 1.

Count your cells and adjust it according to desired concentration like for *Diplonema papillatum* - 2×10^5 cells/ml

Step 2.

Take 96 well plate and add 200ml of 1X sterile PBS in all the outer wells like starting from A -H- 12.

Step 3.

Prepare your drugs stocks for desired concentration.

Step 4.

Add 100ul of media in all the wells

Step 5.

Add 16ul of drug and 184ul of media in the first well and mix it properly and dilute the drug across the plate by transfer 100ul of media containing antibiotics to rest of the wells and discard the last 100ul

Step 6.

Add 200ul of cells to each well starting from last well with lowest antibiotic concentration and now a final concentration of cells are 1×10^5 cells/ml.

Step 7.

Incubate the cells in the wet chamber for 2 days at 27°C.

Step 8.

Observe the cells every day to check whether they are happy or dying.

Step 9.

Add 40ul of Alamar blue assay solution or Resazurin cells by dissolving in 1.25mg/ml 1X PBS, pH should be 7.4, when cells look like they are dying

Step 10.

Incubate them for 17h for minimum and 24h maximum.

Step 11.

Read the fluorescence by using a fluorescence excitation wavelength of 540-570nm.

Step 12.

Analyze the data using GraphPad Prism software using nonlinear regression and sigmoidal dose -response analysis
