IC50 antimicrobial assay
Benzimidazole

Protocol

1. Prepare 10 mL of antimicrobial stock solution (250 ug / mL). Therefore, you must weight 2.5 mg and dilute them in 10 mL of sterile MilliQ water.
2. Label tubes with numbers that go from 1 - 11 for your dilutions. In your first tube, transfer 2 mL of your stock solution.
3. Add 1 mL of sterile MilliQ water into the rest of the tubes (2 - 11).
4. Transfer 1 mL of tube 1 into tube 2 and mix well (vortex if necessary).
5. Transfer 1 mL of tube 2 into tube 3 and mix well (vortex if necessary). Repeat this procedure until you reach tube 11.
6. Discard 1 mL from tube 11.
7. Each concentration will be passage to a 96 well plate. Per concentration, 6 wells of 100 uL each will be prepared. Once in the plate, each of the 6 wells of each dilution will be mixed with 8 x 10^4 resuspended in 50 uL of Tween 20 and 50 uL of sterile PDA media. NOTE: the final concentration in the well will be of 2 x 10^4 CFU / mL
8. The stock solution will be used as a positive control. As it has been proven that at that concentration there must be a 100% of inhibition of the fungi.
9. A negative control of the fungi growing without inhibitor is also recommended. The fungi spores concentrations and dilutions should be respected. Particularly, in this experiment, the negative control would be a mixture of 50 uL of concentrated spores, 50 uL of PDA media diluted in 100 uL of sterile water. (See reference Figure 1 to understand).
10. A negative control for contamination should also be considered; meaning just PDA, Tween 20 and sterile water should be plated. Particularly, in this experiment, this negative control would be a mixture of 100 uL of PDA media diluted in 100 uL of sterile water. (See reference Figure 1 to understand).
11. Measurements of Absorbance should be made according to the fungi in study. Particularly, in this experiment, measurements were made twice a day (in the morning and afternoon) for 5 days at A_{405}.

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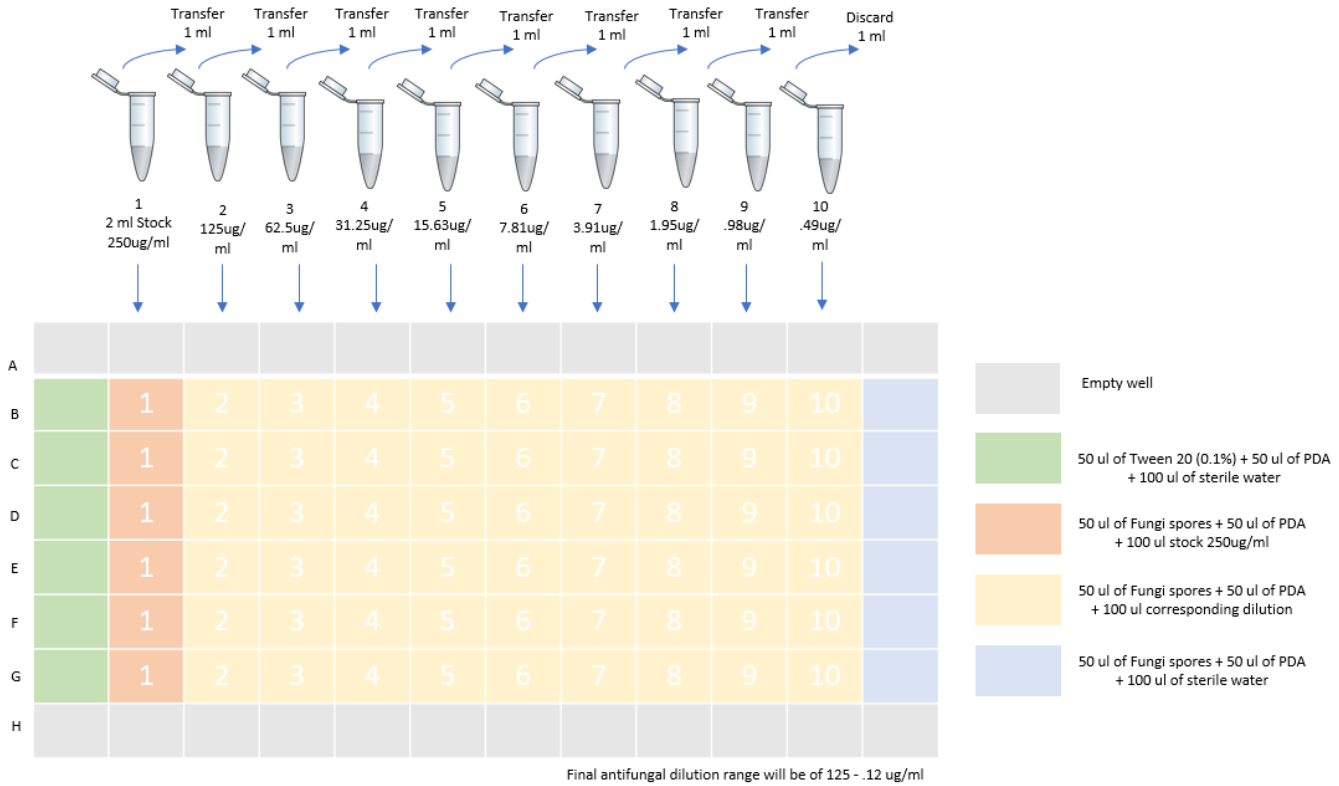

Figure 1. Recommended distribution in a 96 well plate for the IC50 assay.