Schematic Outlining For Preparing Antibiotics for Agar-Dilution: From Stock to Working Concentration

Note: Labs must first make a 100x GEN stock solution consisting of 14 ml GEN at 6400 µg/ml

Transfer 13 ml 100x GEN stock (1:10 dilution)

117 ml sterile distilled water

Transfer 65 ml 1:2 dilution

Final Concentration: 640 µg/ml
Final Volume: 65 ml

65 ml sterile distilled water

Transfer 65 ml 1:2 dilution

Final Concentration: 320 µg/ml
Final Volume: 65 ml

65 ml sterile distilled water

Transfer 65 ml 1:2 dilution

Final Concentration: 160 µg/ml
Final Volume: 65 ml

Continue 1:2 dilutions until 7th flask contains 130 ml of [GEN]=10 µg/ml

Note: One set of GEN plates contains 7 dilutions of GEN and one control plate (0 µg/ml GEN)
Start

Add 21.6 g of GC base medium to 600 ml deionized water

Repeat until 8 flasks are filled with 600 ml of GC base medium

Mix, boil, and autoclave flasks

Add 60 ml of one GEN working concentration dilution to one flask

Add 6 ml of reconstituted IsoVitaleX to each flask

Cool to ~50°C in water-bath

Repeat until all 7 GEN concentrations (10-640 µg/ml) are combined to 7 of 8 flask of GC base medium*

Swirl to mix; pour plates (~25 ml per plate)

End

*one flask will be used for control plates which have no antibiotic

When done, this will make 24 sets of GEN plates with 8 concentrations per set