

4.

CaCl is added to all wells after a two-hour anaerobic incubation from a sterile 1 M stock solution to a final concentration of 20 mM and the chamber is incubated for 15-20 hours at 25°C under 80% N₂, 15% CO₂ and 5% H,



6.

the total volume of precipitate in each field of view is determined from the images by comparing the crystals in the images with an image of a crystal with a defined unit volume

the average volume for a given condition (controls, uninhibited and inhibited bacteria) is then calculated by averaging the volume of precipitate from 10 independent fields of view

З.

the cells are anaerobically resuspended in fresh sterile medium in the 8-well coverslip bottom chambers (0.5 ml original culture per 0.4 ml fresh medium in each well)

TOP VIEW



5.

nigericin added to resuspended G20

0.04 mM

to final concentration of

the precipitate on the bottom of duplicate wells is imaged by the inverted microscope with an attached camera

SIDE VIEW



10x objective transmitted light