

Main sol. 1267b

Solution A	901.00	ml
Solution B	1.00	ml
Solution C	100.00	ml

1. Preparation of the bottom layer: Mix 1 volume of solution A (except NaHCO_3 and agarose) with 1 volume of solution C and add 1% (w/v) agarose type 1, low EEO. After autoclaving, aseptically fill 1.2 ml of the suspension in sterile screw capped tubes (10 ml total volume). The bottom layer solidifies in approx. 30 min.
2. Preparation of the top layer: Add bicarbonate and low melt agarose to solution A and autoclave. Let the sterile solution cool to 40°C and add 10.00 ml/l of solution B. Sparge solution with sterile 100% CO_2 gas until a pH of 6.1 - 6.4 is reached. Then, aseptically pipette aliquots of 6.0 ml over the bottom layer of each tube and let medium equilibrate for at least three hours, but not longer than 12 hours.
3. Inoculation: Inoculate the semisolid top layer with a pipette that is inserted just above the FeS layer; the pipette tip is drawn upward as the inoculum is dispensed.