1267. FERRIPHASELUS (ES) MEDIUM

Solution A:

NH_4Cl	1.00	g
$MgSO_4$ x 7 H_2O	0.20	g
$CaCl_2$ x 2 H_2O	0.10	g
K_2HPO_4	0.05	g
Trace elements (see medium 141)	1.00	ml
MES (SIGMA, for top layer)	1.95	g
$NaHCO_3$ (for top layer)	0.50	g
Low melt agarose (for top layer)	1.50	g
Distilled water	900.00	ml

Solution B:

Vitamin solution (see medium 141)	10.00	ml

Sterilize by filtration and store at 4°C in the dark.

Solution C:

$FeSO_4$ x 7 H_2O	15.40	g
Na_2S x 9 H_2O	12.30	g
Distilled water	100.00	ml

Heat distilled water to 50°C in a 250 ml beaker with a stir bar present. While rapidly stirring the water, add the ferrous sulfate followed immediately by the sodium sulfide. The formed black FeS sludge is decanted into a glass bottle that can be stoppered. The FeS is allowed to settle for several hours and then the overlying water is decanted and replaced. This procedure is repeated at least five times to wash the FeS. After washing, the pH of the FeS solution should be close to neutrality. The FeS suspension can be kept in closed bottles or tubes under a nitrogen atmosphere for at least three months.

Preparation of the bottom layer: Mix 1 volume of *solution A* with 1 volume of *solution C* and add 1% (w/v) agarose type 1, low EEO. After autoclaving, aseptically fill 1 ml of the suspension in sterile Hungate-type tubes (15 ml total volume). The bottom layer solidifies in approx. 30 min.

Preparation of the top layer: Add MES buffer, 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 CO_2 gas until a pH of 6.1 – 6.4 is reached. Then, aseptically pipette aliquots of 5.0 ml over the bottom layer of each tube under 100% N_2 gas atmosphere and let medium equilibrate for at least three hours, but not longer than 12 hours.

Continued on next page

Inoculation: Inoculate the semisolid top layer under a stream of 80% N_2 and 20% CO_2 gas mixture using a sterile Pasteur pipette that is inserted just above the FeS layer; the pipette tip is drawn upward as the inoculum is dispensed. After inoculation close the tube and add 1.00 ml of sterile air.
Note: Some hints on the inoculation of Hungate-tubes under anoxic conditions can be found in the Video tutorial on the „Proper Handling of the Double-Vial Glass Ampoules (anaerobe)“ (https://www.dsmz.de/support/video-tutorials.html).