

1304. SULFURITALEA MEDIUM

MgCl ₂ x 6 H ₂ O	0.20	g
CaCl ₂ x 2 H ₂ O	0.10	g
NaNO ₃	1.70	g
(NH ₄) ₂ SO ₄	0.10	g
KH ₂ PO ₄	0.10	g
KCl	0.10	g
Trace element solution SL-10 (see medium 320)	1.00	ml
Selenite-tungstate solution (see medium 385)	1.00	ml
Na ₂ S ₂ O ₃ x 5 H ₂ O	0.40	g
NaHCO ₃	5.00	g
Vitamins solution (see medium 141)	1.00	ml
Distilled water	1000.00	ml

Dissolve ingredients except trace elements, thiosulfate, bicarbonate and vitamins, then sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 – 45 min to remove dissolved oxygen and to saturate the solution with CO₂. Dispense solution under 80% N₂ and 20% CO₂ gas atmosphere in vials suitable for anaerobic cultures (e.g., Balch-type tubes) to 30% of volume. Close vials with butyl rubber septa and autoclave. Add trace element solution, selenite-tungstate solution and vitamins from sterile anoxic stock solutions prepared under 100% N₂ gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. The vitamin solution should be sterilized by filtration. Adjust pH of the complete medium to 6.7 - 7.0, if necessary. After inoculation, add 1 bar overpressure of sterile 100% H₂ gas.

Note: Use at least 10% (v/v) as inoculum.