

1058c. THIOHALORHABDUS DENITRIFICANS MEDIUM

NaCl	180.00	g
K ₂ HPO ₄	1.50	g
NH ₄ Cl	0.50	g
Trace element solution (see medium 1369)	1.00	ml
CaCl ₂ x 2 H ₂ O	0.05	g
MgSO ₄ x 7 H ₂ O	0.50	g
Na ₂ S ₂ O ₃ x 5 H ₂ O	5.00	g
NaHCO ₃	5.00	g
Seven vitamins solution (see medium 503)	1.00	ml
Distilled water	1000.00	ml

Dissolve sodium chloride, potassium hydrogenphosphate and ammonium chloride, then sparge solution with 80% N₂ and 20% CO₂ gas mixture for at least 30 – 45 min to remove dissolved oxygen and to saturate the solution with CO₂. Dispense solution in vials suitable for anaerobic cultures (e.g. Balch-type tubes) to 30% volume under 80% N₂ and 20% CO₂ gas atmosphere. Close vials with butyl rubber septa and autoclave. Add trace elements, calcium chloride, magnesium sulfate, thiosulfate, bicarbonate and vitamins from sterile stock solutions and adjust pH of the medium to 7.5 - 7.8 using a sterile stock solution of sodium carbonate (5% w/v). After inoculation, add sterile air (with hypodermic needle through the rubber closure) to a concentration of ca. 4% (v/v) O₂ (e.g., add 7 ml of air to a Balch-type tube with a total volume of 27 ml).

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