

## 1058c: THIOHALORHABDUS DENITRIFICANS MEDIUM

Final pH: 7.5 - 7.8

Final volume: 1002 ml

NaCl	180.00	g
K <sub>2</sub> HPO <sub>4</sub>	1.50	g
NH <sub>4</sub> Cl	0.50	g
<b>Trace elements solution (Pfennig, 1965)</b>	1.00	ml
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.05	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	0.50	g
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> x 5 H <sub>2</sub> O	5.00	g
NaHCO <sub>3</sub>	5.00	g
<b>Seven vitamins solution</b>	1.00	ml
Distilled water	1000.00	ml

1. Dissolve sodium chloride, potassium hydrogenphosphate and ammonium chloride, then sparge solution with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for at least 30 - 45 min to remove dissolved oxygen and to saturate the solution with CO<sub>2</sub>. Dispense solution in vials suitable for anaerobic cultures (e.g. Balch-type tubes) to 30% volume under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere. Close vials with butyl rubber septa and autoclave. Add trace elements, calcium chloride, magnesium sulfate, thiosulfate, and vitamins from sterile stock solutions prepared under 100% N<sub>2</sub> gas and bicarbonate from a sterile stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. Thiosulfate and vitamins are sterilized by filtration. Adjust pH of the medium to 7.5 - 7.8 using a sterile stock solution of sodium carbonate (5% w/v).

2. After inoculation, add sterile air (with hypodermic needle through the rubber closure) to a concentration of ca. 4% (v/v) O<sub>2</sub> (e.g., add 7 ml of air to a Balch-type tube with a total volume of 27 ml).

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

### Trace elements solution (Pfennig, 1965) (from medium 1369)

EDTA	5.00	g
FeSO <sub>4</sub> x 7 H <sub>2</sub> O	2.20	g
ZnSO <sub>4</sub> x 7 H <sub>2</sub> O	0.10	g
MnCl <sub>2</sub> x 4 H <sub>2</sub> O	0.03	g
H <sub>3</sub> BO <sub>3</sub>	0.03	g
CoCl <sub>2</sub> x 6 H <sub>2</sub> O	0.20	g
CuCl <sub>2</sub> x 2 H <sub>2</sub> O	0.03	g
NiCl <sub>2</sub> x 6 H <sub>2</sub> O	0.03	g
Na <sub>2</sub> MoO <sub>4</sub> x 2 H <sub>2</sub> O	0.03	g

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Distilled water	1000.00	ml
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pH 3.0-4.0

### **Seven vitamins solution** (from medium 503)

Vitamin B <sub>12</sub>	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H <sub>2</sub> O	200.00	mg
Distilled water	1000.00	ml