

## 1058d: THIOHALOMONAS DENITRIFICANS MEDIUM

NaCl	120.00	g
K <sub>2</sub> HPO <sub>4</sub>	1.50	g
NH <sub>4</sub> Cl	0.50	g
KNO <sub>3</sub>	1.00	g
<b>Trace elements solution (Pfennig &amp; Lippert,1966)</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, ammonium chloride and potassium nitrate, 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. Dispense solution under same gas atmosphere in vials suitable for anaerobic cultures (e.g. Balch-type tubes) to 50% volume, close vials with butyl rubber septa and autoclave. Add trace elements, calcium chloride, magnesium sulfate, thiosulfate, and vitamins from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. Thiosulfate and vitamins should be sterilized by filtration. Adjust pH of the complete medium to 7.5 - 7.8 using a sterile anoxic stock solution of sodium carbonate (5% w/v) prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere.

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

For DSM 16925: Reduce amount of thiosulfate to 1.00 g/l.

### Trace elements solution (Pfennig & Lippert,1966) (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
Distilled water	1000.00	ml

pH 3.0-4.0

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### 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