

## 1058e: THIOHALOBACTER THIOCYANATICUS MEDIUM

NaCl	60.00	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 &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 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 under air atmosphere in serum vials to 10% of volume, seal with butyl rubber stoppers 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 anoxic stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub>. Thiosulfate and vitamins are sterilized by filtration. Adjust pH of the medium to 7.5 using a sterile stock solution of sodium carbonate (5% w/v). Incubate with shaking.

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

### 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

### Seven vitamins solution (from medium 503)

Vitamin B <sub>12</sub>	100.00	mg
p-Aminobenzoic acid	80.00	mg

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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