

## 1092: THERMOSULFIDIBACTER MEDIUM

Sea Salt (SIGMA)	30.00	g
<b>Wolfe's mineral elixir</b>	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Sulfur (powdered)	3.00	g
NaHCO <sub>3</sub>	2.50	g
Yeast extract	2.00	g
<b>Wolin's vitamin solution (10x)</b>	2.00	ml
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except sulfur, bicarbonate, yeast extract, vitamins and sulfide) and sparge medium with 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic serum vials (e.g., Balch-type tubes) containing already the appropriate amount of sulfur, only to 30% of their volume to allow for a large headspace. Autoclave at **110°C** for 20 min. Add yeast extract, vitamins and sulfide 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. The vitamin solution should be sterilized by filtration. Adjust pH of the complete medium to 7.0-7.2.
2. After inoculation pressurize vessels with sterile 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture to 2 bar overpressure.

### Wolfe's mineral elixir (from medium 792)

MgSO <sub>4</sub> x 7 H <sub>2</sub> O	30.00	g
MnSO <sub>4</sub> x H <sub>2</sub> O	5.00	g
NaCl	10.00	g
FeSO <sub>4</sub> x 7 H <sub>2</sub> O	1.00	g
CoCl <sub>2</sub> x 6 H <sub>2</sub> O	1.80	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	1.00	g
ZnSO <sub>4</sub> x 7 H <sub>2</sub> O	1.80	g
CuSO <sub>4</sub> x 5 H <sub>2</sub> O	0.10	g
AlK(SO <sub>4</sub> ) <sub>2</sub> x 12 H <sub>2</sub> O	0.18	g
H <sub>3</sub> BO <sub>3</sub>	0.10	g
Na <sub>2</sub> MoO <sub>4</sub> x 2 H <sub>2</sub> O	0.10	g
(NH <sub>4</sub> ) <sub>2</sub> Ni(SO <sub>4</sub> ) <sub>2</sub> x 6 H <sub>2</sub> O	2.80	g
Na <sub>2</sub> WO <sub>4</sub> x 2 H <sub>2</sub> O	0.10	g
Na <sub>2</sub> SeO <sub>4</sub>	0.10	g
Distilled water	1000.00	ml

First adjust pH to 1.0 with diluted H<sub>2</sub>SO<sub>4</sub>, then add and dissolve the salts.

### Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B <sub>12</sub>	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml