

**711: SPOROBACTER MEDIUM**

NH <sub>4</sub> Cl	1.00	g
K <sub>2</sub> HPO <sub>4</sub>	0.30	g
KH <sub>2</sub> PO <sub>4</sub>	0.30	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	0.20	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.10	g
KCl	0.10	g
NaCl	0.60	g
Yeast extract	0.50	g
Na-acetate x 3 H <sub>2</sub> O	0.50	g
<b>Trace element solution SL-10</b>	1.50	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na <sub>2</sub> CO <sub>3</sub>	1.50	g
3,4,5-Trimethoxycinnamic acid (TMC)	1.19	g
<b>Wolin's vitamin solution (10x)</b>	1.00	ml
L-Cysteine HCl x H <sub>2</sub> O	0.30	g
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate, vitamins, TMC and reducing agents) and sparge medium with 80% N<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 Hungate-type tubes or serum vials and autoclave. After autoclaving add vitamins, TMC, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas and carbonate from a sterile anoxic stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere. The vitamin solution should be sterilized by filtration. The stock solution of TMC (6% w/v) should be neutralized with NaOH prior to sterilization by filtration. Adjust pH of complete medium to 7.0, if necessary.

**Trace element solution SL-10 (from medium 320)**

HCl (25%)	10.00	ml
FeCl <sub>2</sub> x 4 H <sub>2</sub> O	1.50	g
ZnCl <sub>2</sub>	70.00	mg
MnCl <sub>2</sub> x 4 H <sub>2</sub> O	100.00	mg
H <sub>3</sub> BO <sub>3</sub>	6.00	mg
CoCl <sub>2</sub> x 6 H <sub>2</sub> O	190.00	mg
CuCl <sub>2</sub> x 2 H <sub>2</sub> O	2.00	mg
NiCl <sub>2</sub> x 6 H <sub>2</sub> O	24.00	mg
Na <sub>2</sub> MoO <sub>4</sub> x 2 H <sub>2</sub> O	36.00	mg
Distilled water	990.00	ml

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First dissolve  $\text{FeCl}_2$  in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

### **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