

**1699: SULFURIMONAS CRATERIS MEDIUM**

<b>Solution A</b>	981.00	ml
<b>Solution B</b>	25.00	ml
<b>Solution C</b>	1.00	ml

Dissolve compounds of solution A except sulfur and sparge it with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 - 45 min to make it anoxic. Distribute under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere into anoxic Hungate-type tubes or serum vials that contain the appropriate amount of sulfur and autoclave at **110°C** for 20 min. Solution B is autoclaved under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere. Solution C is prepared under 100% N<sub>2</sub> gas and sterilized by filtration. To complete the medium appropriate amounts of solutions B and C are added to the sterile solution A in the sequence as indicated. Adjust pH of complete medium to 7.5 - 8.0, if necessary.

**Solution A**

KH <sub>2</sub> PO <sub>4</sub>	0.20	g
NH <sub>4</sub> Cl	0.25	g
NaCl	10.00	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	0.40	g
KCl	0.50	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.15	g
NaNO <sub>3</sub>	0.85	g
<b>Modified Wolin's mineral solution</b>	1.00	ml
Sulfur (powdered)	5.00	g
Distilled water	980.00	ml

**Solution B**

Na <sub>2</sub> CO <sub>3</sub>	1.25	g
Distilled water	25.00	ml

**Solution C**

<b>Wolin's vitamin solution (10x)</b>	1.00	ml
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**Modified Wolin's mineral solution** (from medium 141)

Nitrilotriacetic acid	1.50	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	3.00	g
MnSO <sub>4</sub> x H <sub>2</sub> O	0.50	g
NaCl	1.00	g
FeSO <sub>4</sub> x 7 H <sub>2</sub> O	0.10	g

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CoSO <sub>4</sub> x 7 H <sub>2</sub> O	0.18	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.10	g
ZnSO <sub>4</sub> x 7 H <sub>2</sub> O	0.18	g
CuSO <sub>4</sub> x 5 H <sub>2</sub> O	0.01	g
AlK(SO <sub>4</sub> ) <sub>2</sub> x 12 H <sub>2</sub> O	0.02	g
H <sub>3</sub> BO <sub>3</sub>	0.01	g
Na <sub>2</sub> MoO <sub>4</sub> x 2 H <sub>2</sub> O	0.01	g
NiCl <sub>2</sub> x 6 H <sub>2</sub> O	0.03	g
Na <sub>2</sub> SeO <sub>3</sub> x 5 H <sub>2</sub> O	0.30	mg
Na <sub>2</sub> WO <sub>4</sub> x 2 H <sub>2</sub> O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

### 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