

**1053: SULFURIMONAS PARALVINELLA MEDIUM**

NaCl	20.00	g
K <sub>2</sub> HPO <sub>4</sub>	0.09	g
KH <sub>2</sub> PO <sub>4</sub>	0.07	g
CaCl <sub>2</sub>	0.80	g
NH <sub>4</sub> Cl	0.25	g
NaNO <sub>3</sub>	1.00	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	4.00	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	3.00	g
KCl	0.33	g
Fe <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub> x H <sub>2</sub> O	0.01	g
<b>Modified Wolin's mineral solution</b>	10.00	ml
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> x 5 H <sub>2</sub> O	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Sulfur (powdered)	10.00	g
NaHCO <sub>3</sub>	1.00	g
<b>Wolin's vitamin solution (10x)</b>	1.00	ml
Distilled water	1000.00	ml

1. Dissolve ingredients (except sulfur, bicarbonate and vitamins), then sparge medium with 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 - 45 min to make it anoxic. Adjust pH to 6.8 with NaOH and dispense under 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere into anoxic Hungate-type tubes or serum vials (up to a volume of 20%) that contain already the appropriate amount of sulfur. Autoclave at a temperature of **110°C** for 20 min. Add vitamins and bicarbonate to the autoclaved medium from sterile anoxic stock solutions. The solution of vitamins is sterilized by filtration and stored under 100% N<sub>2</sub> gas, whereas the solution of bicarbonate is prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture and autoclaved. Adjust the pH of the complete medium to 6.5.

2. After inoculation pressurize vessels to 2 bar overpressure with sterile 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture.

**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
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

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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