

## Main sol. 404

<b>Mineral solution 1</b>	40.00	ml
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Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> x 7 H <sub>2</sub> O (0.1% w/v)	2.00	ml
NiCl <sub>2</sub> x 6 H <sub>2</sub> O (0.1% w/v)	0.20	ml
<b>Modified Wolin's mineral solution</b>	10.00	ml
Na-acetate x 3 H <sub>2</sub> O	3.00	g
Yeast extract (OXOID)	1.00	g
Trypticase peptone (BD BBL)	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
NaHCO <sub>3</sub>	3.00	g
Methanol (50% v/v)	10.00	ml
<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	900.00	ml

1. Dissolve ingredients (except bicarbonate, methanol, vitamins, cysteine and sulfide), 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. Add and dissolve bicarbonate and adjust pH to 7.0, then distribute under 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. After sterilization add methanol (50% v/v), vitamins, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas atmosphere. Vitamins are sterilized by filtration. Adjust pH of complete medium to 7.0 - 7.2.
2. After inoculation, pressurize culture bottles with sterile 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture to 2 bar overpressure.