



## Main sol. 579

Fe(III) citrate	13.70	g
NH <sub>4</sub> Cl	1.50	g
NaH <sub>2</sub> PO <sub>4</sub>	0.60	g
KCl	0.10	g
Na-acetate	2.50	g
<b>Modified Wolin's mineral solution</b>	10.00	ml
Na <sub>2</sub> WO <sub>4</sub> x 2 H <sub>2</sub> O (0.1% w/v)	0.25	ml
NaHCO <sub>3</sub>	2.50	g
<b>Wolin's vitamin solution (10x)</b>	1.00	ml
Distilled water	1000.00	ml

1. First dissolve ferric citrate in water by heating and adjust to pH 6.0, then add other medium ingredients except bicarbonate and vitamins. Sparge medium with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 - 45 min to make it anoxic, then add and dissolve bicarbonate and adjust pH to 6.8. Thereafter, dispense medium under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add vitamins to the sterile medium from an anoxic stock solution prepared under 100% N<sub>2</sub> gas and sterilized by filtration. Adjust pH of complete medium to 6.7 - 7.0, if necessary.

2. Note: Use 5 - 10% (v/v) inoculum.