

## 526. CLOSTRIDIUM VIRIDE MEDIUM

5-Aminovalerate	15.00	g
K <sub>2</sub> HPO <sub>4</sub>	8.70	g
Yeast extract	7.50	g
Trace elements solution VR (see below)	4.00	ml
Na-resazurin solution (0.1% w/v)	0.50	ml
NaHCO <sub>3</sub>	2.50	g
L-Cysteine-HCl x H <sub>2</sub> O	0.25	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate and cysteine), boil medium for 1 min, then cool to room temperature under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. Add solid bicarbonate and cysteine, dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving adjust pH of medium to 7.6 - 7.8 using a sterile anoxic stock solution of Na<sub>2</sub>CO<sub>3</sub> (5% w/v) prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere.

### *Trace elements solution VR:*

Nitrilotriacetic acid (NTA)	5.00	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	24.00	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.50	g
FeSO <sub>4</sub> x 7 H <sub>2</sub> O	0.50	g
NiCl <sub>2</sub> x 6 H <sub>2</sub> O	0.25	g
ZnSO <sub>4</sub> x 7 H <sub>2</sub> O	0.44	g
MnSO <sub>4</sub> x H <sub>2</sub> O	0.25	g
CoCl <sub>2</sub> x 6 H <sub>2</sub> O	0.25	g
VO <sub>2</sub> SO <sub>4</sub> x 5 H <sub>2</sub> O	0.25	g
Na <sub>2</sub> MoO <sub>4</sub> x 2 H <sub>2</sub> O	0.25	g
CuSO <sub>4</sub> x 5 H <sub>2</sub> O	0.12	g
Distilled water	1000.00	ml

Dissolve mineral salts in 700 ml water acidified with 2 ml of conc. HCl. Separately dissolve 5.00 g of NTA in 300 ml water add 10 N NaOH until all the NTA has dissolved and the pH is 7.0. While stirring, slowly add the NTA solution to the salt solution. Stir until the solution is almost clear. Store cool and dark.