

526. CLOSTRIDIUM VIRIDE MEDIUM

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| 5-Aminovaleric acid | 15.00 | g |
| K ₂ HPO ₄ | 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 ₃ | 2.50 | g |
| L-Cysteine-HCl x H ₂ O | 0.25 | g |
| Distilled water | 1000.00 | ml |

Dissolve ingredients (except bicarbonate and cysteine), adjust pH to 7.0 with NaOH and sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 – 45 min. Add and dissolve 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 Na₂CO₃ (5% w/v) prepared under 80% N₂ and 20% CO₂ gas atmosphere.

Trace elements solution VR:

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|---|---------|----|
| Nitrilotriacetic acid (NTA) | 5.00 | g |
| MgSO ₄ x 7 H ₂ O | 24.00 | g |
| CaCl ₂ x 2 H ₂ O | 0.50 | g |
| FeSO ₄ x 7 H ₂ O | 0.50 | g |
| NiCl ₂ x 6 H ₂ O | 0.25 | g |
| ZnSO ₄ x 7 H ₂ O | 0.44 | g |
| MnSO ₄ x H ₂ O | 0.25 | g |
| CoCl ₂ x 6 H ₂ O | 0.25 | g |
| VO ₂ SO ₄ x 5 H ₂ O | 0.25 | g |
| Na ₂ MoO ₄ x 2 H ₂ O | 0.25 | g |
| CuSO ₄ x 5 H ₂ 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.