

Main sol. 1022

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| 4-Hydroxybenzoic acid | 0.45 | g |
| K ₂ HPO ₄ | 0.40 | g |
| NH ₄ Cl | 0.40 | g |
| Yeast extract (BD Bacto) | 5.00 | g |
| Casamino acids (BD Bacto) | 1.00 | g |
| Trace element solution SL-10 | 2.00 | ml |
| Selenite-tungstate solution | 1.00 | ml |
| Sodium resazurin (0.1% w/v) | 0.50 | ml |
| NaHCO ₃ | 4.00 | g |
| C. sporogenes supernatant | 350.00 | ml |
| MgCl ₂ x 6 H ₂ O | 0.08 | g |
| CaCl ₂ x 2 H ₂ O | 0.06 | g |
| Wolin's vitamin solution (10x) | 1.00 | ml |
| Distilled water | 650.00 | ml |

1. Dissolve ingredients (except bicarbonate, C. sporogenes supernatant, magnesium chloride, calcium chloride, and vitamins), adjust pH to 7.0 - 7.5 and boil medium for 1 min, then cool to room temperature under 80% N₂ and 20% CO₂ gas mixture. Dissolve solid bicarbonate, adjust pH to 7.8, dispense the solution under 80% N₂ and 20% CO₂ gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving add the appropriate amount of sterile and anoxic supernatant of C. sporogenes and complete the medium by adding magnesium chloride, calcium chloride and vitamins (sterilized by filtration) from sterile anoxic stock solutions prepared under 100% N₂ gas. The final pH of the medium should be 7.5 - 8.0.
2. It may be necessary to add 10 - 20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution, freshly prepared under N₂ gas and filter-sterilized), if the medium is not completely reduced after inoculation.
3. Note: For transfers use 10% (v/v) inoculum.