

Main sol. 1022

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.