

711. SPOROBACTER MEDIUM

NH ₄ Cl	1.00	g
K ₂ HPO ₄	0.30	g
KH ₂ PO ₄	0.30	g
MgCl ₂ x 6 H ₂ O	0.20	g
CaCl ₂ x 2 H ₂ O	0.10	g
KCl	0.10	g
NaCl	0.60	g
Yeast extract	0.50	g
Na-acetate x 3 H ₂ O	0.50	g
Trace element solution SL-10 (see medium 320)	1.50	ml
Na-resazurin solution (0.1% w/v)	0.50	ml
Na ₂ CO ₃	1.50	g
Vitamin solution (see medium 141)	10.00	ml
<i>trans</i> -3,4,5-trimethoxycinnamate (TMC)	1.19	g
L-Cysteine-HCl x H ₂ O	0.30	g
Na ₂ S x 9 H ₂ O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate, vitamins, TMC and reducing agents) and sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving add vitamins, TMC, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas and carbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas atmosphere. The vitamin solution should be sterilized by filtration. The stock solution of TMC (6% w/v) should be neutralized with NaOH prior to sterilization by filtration. Adjust pH of complete medium to 7.0, if necessary.