



Main sol. 1277

NaCl	0.25	g
MgCl ₂ x 6 H ₂ O	0.20	g
CaCl ₂ x 2 H ₂ O	0.10	g
NaNO ₃	1.70	g
(NH ₄) ₂ SO ₄	0.10	g
KH ₂ PO ₄	0.10	g
KCl	0.10	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Na ₂ CO ₃	1.50	g
Na ₂ S ₂ O ₃ x 5 H ₂ O	2.50	g
Wolin's vitamin solution (10x)	1.00	ml
Distilled water	1000.00	ml

1. Dissolve ingredients (except carbonate, thiosulfate and vitamins) 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 to 30% volume and autoclave. Add thiosulfate and vitamins 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 mixture. Stock solutions of thiosulfate and vitamins should be sterilized by filtration. Adjust pH of complete medium to 7.5, if necessary.
2. After inoculation add sterile air in an amount equivalent to 1% (v/v) of the total volume of the culture vessel.