



Main sol. 1584

NaCl	8.80	g
KCl	0.34	g
NH ₄ Cl	0.25	g
MgCl ₂ x 6 H ₂ O	4.84	g
CaCl ₂ x 2 H ₂ O	0.15	g
K ₂ HPO ₄	0.15	g
Fe(NH ₄) ₂ (SO ₄) ₂ (0.1% w/v)	3.00	ml
NiCl ₂ x 6 H ₂ O (0.1% w/v)	9.00	ml
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Na ₂ CO ₃	1.50	g
Na ₂ -fumarate	8.00	g
Wolin's vitamin solution (10x)	1.00	ml
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate, fumarate and vitamins) and sparge medium with 80% N₂ and 20% CO₂ gas mixture to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving complete the medium by adding fumarate 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 fumarate and vitamins are sterilized by filtration. Adjust pH of the complete medium to 7.0 - 7.2, if necessary.