

Main sol. 311c

NH ₄ Cl	0.50	g
MgSO ₄ x 7 H ₂ O	0.50	g
CaCl ₂ x 2 H ₂ O	0.25	g
NaCl	2.25	g
FeSO ₄ x 7 H ₂ O (0.1% w/v in 0.1 N H ₂ SO ₄)	2.00	ml
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Yeast extract	2.00	g
Casitone	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
K ₂ HPO ₄	0.35	g
KH ₂ PO ₄	0.23	g
Na ₂ CO ₃	1.00	g
D-Fructose	5.00	g
Wolin's vitamin solution (10x)	1.00	ml
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 phosphates, carbonate, fructose, vitamins, cysteine and sulfide) 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 phosphates, fructose, vitamins, cysteine and sulfide to the medium 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 fructose and vitamins should be sterilized by filtration. Adjust pH of complete medium to pH 6.5 - 7.0, if necessary.