

Main sol. 318c

KH ₂ PO ₄	0.30	g
NaCl	0.60	g
MgCl ₂ x 6 H ₂ O	0.10	g
CaCl ₂ x 2 H ₂ O	0.08	g
Trace element solution	10.00	ml
NH ₄ Cl	1.00	g
Yeast extract (OXOID)	0.50	g
Trypticase peptone (BD BBL)	0.50	g
Sodium resazurin (0.1% w/v)	0.50	ml
KHCO ₃	2.50	g
Na-gallate	1.90	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 bicarbonate, vitamins, gallate, cysteine and sulfide, then sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Dispense medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add vitamins, gallate, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. Stock solutions of vitamins and gallate are sterilized by filtration. Prior to use adjust pH of complete medium to 7.3, if necessary.