

Main sol. 318a

HEPES (SIGMA)	11.92	g
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
Sodium resazurin (0.1% w/v)	0.50	ml
KHCO ₃	2.00	g
2-Furoic acid (SIGMA)	2.24	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, furoic acid, cysteine and sulfide and adjust pH of solution to 7.0. 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 furoic acid (stock solution neutralized with NaOH), 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. Prior to use adjust pH of complete medium to 7.0 - 7.2, if necessary.