

Main sol. 1095

NaCl	100.00	g
KH ₂ PO ₄	0.20	g
MgCl ₂	0.10	g
NH ₄ Cl	0.50	g
Yeast extract (OXOID)	10.00	g
Tryptone (BD Bacto)	10.00	g
Trace element solution SL-10	1.00	ml
Na ₂ CO ₃	68.00	g
NaHCO ₃	38.00	g
L-Cysteine HCl x H ₂ O	0.70	g
Wolin's vitamin solution (10x)	1.00	ml
Sucrose	5.00	g
Distilled water	1000.00	ml

Dissolve ingredients (except carbonates, cysteine, sucrose and vitamins), then sparge medium with 100% N₂ gas for 30 - 45 min to make it anoxic. Add carbonate, hydrogencarbonate and cysteine and adjust pH to 8.5. Dispense medium under 100% N₂ gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving add sucrose and vitamins from sterile anoxic stock solutions prepared under 100% N₂ gas and sterilized by filtration.