



## Main sol. 1095

NaCl	100.00	g
KH <sub>2</sub> PO <sub>4</sub>	0.20	g
MgCl <sub>2</sub>	0.10	g
NH <sub>4</sub> Cl	0.50	g
Yeast extract (OXOID)	10.00	g
Tryptone (BD Bacto)	10.00	g
<b>Trace element solution SL-10</b>	1.00	ml
Na <sub>2</sub> CO <sub>3</sub>	68.00	g
NaHCO <sub>3</sub>	38.00	g
L-Cysteine HCl x H <sub>2</sub> O	0.70	g
<b>Wolin's vitamin solution (10x)</b>	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% N<sub>2</sub> gas for 30 - 45 min to make it anoxic. Add carbonate, hydrogencarbonate and cysteine and adjust pH to 8.5. Dispense medium under 100% N<sub>2</sub> 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% N<sub>2</sub> gas and sterilized by filtration.