

Main sol. 826

NH ₄ Cl	1.50	g
Na ₂ HPO ₄	0.60	g
KCl	0.10	g
Na-acetate	0.82	g
Modified Wolin's mineral solution	10.00	ml
NaHCO ₃	2.50	g
Na ₂ -fumarate	8.00	g
Wolin's vitamin solution (10x)	1.00	ml
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, fumarate and vitamins), sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Add bicarbonate and equilibrate the medium with 80% N₂ and 20% CO₂ gas mixture to reach a pH of 6.8, then dispense medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving add fumarate and vitamins from anoxic stock solutions prepared under 100% N₂ gas atmosphere and sterilized by filtration. Check medium pH and adjust to 6.8, if necessary.