



Main sol. 854

NH ₄ Cl	0.33	g
CaCl ₂ x 2 H ₂ O	0.33	g
MgCl ₂ x 6 H ₂ O	0.33	g
KCl	0.33	g
KH ₂ PO ₄	0.33	g
NaCl	25.00	g
MOPS buffer (SIGMA)	3.00	g
Modified Wolin's mineral solution	10.00	ml
Yeast extract	0.10	g
Na-acetate	5.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Sulfur (powdered)	10.00	g
Wolin's vitamin solution (10x)	1.00	ml
Na ₂ S x 9 H ₂ O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except sulfur, vitamins, and sulfide), adjust pH to 5.5 - 6.0, and sparge the medium with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Dispense the medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials that contain already an appropriate amount of sulfur powder. Autoclave 20 min at **110°C**. Prior to use add vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas. Vitamins are sterilized by filtration. Adjust the pH of the complete medium to 6.0 - 6.2, if necessary.