

Main sol. 141a

KCl	0.34	g
MgCl ₂ x 6 H ₂ O	4.00	g
MgSO ₄ x 7 H ₂ O	3.45	g
NH ₄ Cl	0.25	g
CaCl ₂ x 2 H ₂ O	0.14	g
K ₂ HPO ₄	0.14	g
NaCl	18.00	g
Modified Wolin's mineral solution	10.00	ml
Fe(NH ₄) ₂ (SO ₄) ₂ x 6 H ₂ O (0.1% w/v)	2.00	ml
Na-acetate	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H ₂ O	0.50	g
Na ₂ S x 9 H ₂ O	0.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except vitamins, cysteine, and sulfide), sparge medium with 80% H₂ and 20% CO₂ gas mixture to reach a pH below 6 (at least 30 min), then dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. After sterilization add cysteine and sulfide from sterile anoxic stock solutions autoclaved under 100% N₂ gas atmosphere. Vitamins are prepared under 100% N₂ gas atmosphere and sterilized by filtration. Adjust the pH of the final medium to 6.2.

2. For incubation use sterile 80% H₂ and 20% CO₂ gas mixture at two atmospheres of pressure.