Main sol. 141b

KCl	0.34	g
$MgCl_2$ x 6 H_2O	4.00	g
$MgSO_4$ x 7 H_2O	3.45	g
NH_4Cl	0.25	g
$CaCl_2$ x 2 H_2O	0.14	g
K_2HPO_4	0.14	g
NaCl	6.00	g
Modified Wolin's mineral solution	10.00	ml
$Fe(NH_4)_2(SO_4)_2$ x 6 H_2O (0.1% w/v)	2.00	ml
Na-acetate	1.00	g
Yeast extract (OXOID)	2.00	g
Trypticase peptone (BD BBL)	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Wolin's vitamin solution (10x)	1.00	ml
$NaHCO_3$	5.00	g
L-Cysteine HCl x H_2O	0.50	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except bicarbonate, vitamins, cysteine and sulfide), sparge medium with 80% H_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Add and dissolve bicarbonate and adjust pH to 7.0, then dispense medium under 80% H_2 and 20% CO_2 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% N_2 gas atmosphere. Vitamins are prepared under 100% N_2 gas atmosphere and sterilized by filtration. Adjust pH of final medium to 6.8 - 7.0.

2. For incubation use sterile 80% H_2 and 20% CO_2 gas mixture at two atmospheres of pressure.

3. Note: If the medium is being used without overpressure then adjust pH with a small amount of sterile anoxic 1 N HCl, if necessary.