Main sol. 817

$MgCl_2$ x 6 H_2O	2.20	g
NaCl	0.90	g
KCl	17.00	mg
NH_4Cl	12.00	mg
$CaCl_2$ x 2 H_2O (0.1% w/v)	7.00	ml
K_2HPO_4 x 3 H_2O (0.1% w/v)	7.00	ml
$FeCl_3$ (0.01% w/v in 0.2 N HCl)	0.50	ml
Yeast extract (BD Bacto)	1.00	g
Peptone (BD Bacto)	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	0.50	g
Wolin's vitamin solution (10x)	1.00	ml
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients except carbonate, vitamins and sulfide. Sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. Add vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere. Vitamins should be sterilized by filtration. The pH of the complete medium should be adjusted to 6.5.

2. After inoculation pressurize tubes or bottles (heavy walled) to 2 bar overpressure with sterile 80% N_2 and 20% CO_2 gas mixture.