377b: PYROCOCCUS ST04 MEDIUM

NaCl	13.85	g
$MgSO_4$ x 7 H_2O	3.50	g
$MgCl_2$ x 6 H_2O	2.75	g
KCl	0.33	g
NaBr	0.05	g
H_3BO_3	15.00	mg
SrCl2 x 6 H_2O (0.1% w/v)	7.00	ml
$(NH_4)_2SO_4$	10.00	mg
Citric acid (0.1% w/v)	5.00	ml
KI (0.01% w/v)	0.50	ml
$CaCl_2$ x 2 H_2O	0.75	g
KH_2PO_4	0.50	g
$NiCl_2$ x 6 H_2O (0.1% w/v)	2.00	ml
Modified Wolin's mineral solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Sulfur (powdered)	30.00	g
Peptone (BD Bacto)	5.00	g
Yeast extract (OXOID)	1.00	g
$NaHCO_3$	2.00	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients except sulfur, peptone, yeast extract, bicarbonate, and sulfide, adjust the pH to 6.8, and sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Distribute medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials that already contain the appropriate amount of sulfur. Sterilize the medium by autoclaving at **110°C** for 20 min. After sterilization add peptone, yeast extract, and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas atmosphere and bicarbonate from a sterile stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Adjust the pH of the complete medium to 6.8, if necessary.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g

$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.