

**377b. PYROCOCCLUS ST04 MEDIUM**

NaCl	13.85	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	3.50	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	2.75	g
KCl	0.33	g
NaBr	0.05	g
H <sub>3</sub> BO <sub>3</sub>	15.00	mg
SrCl <sub>2</sub> x 6 H <sub>2</sub> O solution (0.1% w/v)	7.00	ml
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	10.00	mg
Citric acid solution (0.1% w/v)	5.00	ml
KI solution (0.01% w/v)	0.50	ml
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.75	g
KH <sub>2</sub> PO <sub>4</sub>	0.50	g
NiCl <sub>2</sub> x 6 H <sub>2</sub> O (0.1% w/v)	2.00	ml
Trace element solution (see medium 141)	10.00	ml
Peptone (BD Bacto)	5.00	g
Yeast extract (OXOID)	1.00	g
Na-resazurin solution (0.1% w/v)	0.50	ml
Sulfur, powdered	30.00	g
NaHCO <sub>3</sub>	2.00	g
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients except sulfur, bicarbonate and sulfide, adjust the pH to 6.8, and sparge medium with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 – 45 min to make it anoxic. Distribute medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials that contain already the appropriate amount of sulfur. Sterilize medium by heating cultivation vessels in a boiling water bath for 1 - 2 hours on each of 3 successive days. After sterilization add sulfide from a sterile anoxic stock solution prepared under 100% N<sub>2</sub> gas atmosphere and bicarbonate from a sterile stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. Adjust pH of complete medium to 6.8, if necessary.