

377. PYROCOCCLUS MEDIUM

NaCl	13.85	g
MgSO ₄ x 7 H ₂ O	3.50	g
MgCl ₂ x 6 H ₂ O	2.75	g
KCl	0.33	g
NaBr	0.05	g
H ₃ BO ₃	15.00	mg
SrCl ₂ x 6 H ₂ O solution (0.1% w/v)	7.00	ml
(NH ₄) ₂ SO ₄	10.00	mg
Citric acid solution (0.1% w/v)	5.00	ml
KI solution (0.01% w/v)	0.50	ml
CaCl ₂ x 2 H ₂ O	0.75	g
KH ₂ PO ₄	0.50	g
NiCl ₂ x 6 H ₂ 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
Na ₂ S x 9 H ₂ O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients except sulfur and sulfide, adjust the pH to 6.5, and sparge medium with 100% N₂ gas 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. Add sulfide from a neutralized stock solution (see medium 29) prepared under 100% N₂ gas atmosphere.