

491. HYPERTHERMUS BUTYLICUS MEDIUM

NaCl	17.00	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
NH ₄ Cl	0.50	g
Citric acid solution (0.1% w/v)	5.00	ml
KI solution (0.1% w/v)	2.50	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 solution (0.1% w/v)	2.00	ml
Trace element solution (see medium 141)	10.00	ml
Tryptone (BD BBL)	6.00	g
Na-resazurin solution (0.1% w/v)	0.50	ml
Sulfur, powder	6.00	g
Na ₂ S x 9 H ₂ O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients except sulfur and sulfide and adjust pH to 6.0 with sulfuric acid. Sparge medium with 100% N₂ gas for 30 – 45 min to make it anoxic, dispense under the same gas atmosphere into anoxic Hungate-type tubes or serum vials containing the appropriate amount of sulfur. Sterilize medium by heating for 2 – 3 hours in a boiling water bath on each of 3 successive days. Before use reduce the medium by adding sulfide from a sterile anoxic stock solution prepared under 100% N₂ gas atmosphere. Check pH and adjust to pH 6.5 - 7.0, if necessary. After inoculation pressurize culture vessels to 1 bar overpressure with a sterile 80% H₂ and 20% CO₂ gas mixture.