491: HYPERTHERMUS BUTYLICUS MEDIUM

NaCl	17.00	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
NH_4Cl	0.50	g
Citric acid (0.1% w/v)	5.00	ml
KI (0.1% w/v)	2.50	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
Tryptone (BD BBL)	6.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Sulfur (powder)	6.00	g
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

1. Dissolve ingredients except sulfur and sulfide and adjust pH to 6.0 with sulfuric acid. Sparge medium with 100% N_2 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, only to 30% of their volume to allow for a large headspace. 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% N_2 gas atmosphere. Check pH and adjust to pH 6.5 - 7.0, if necessary.

2. After inoculation pressurize culture vessels to 1 bar overpressure with a sterile 80% H_2 and 20% CO_2 gas mixture.

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.