1662: ALKALIPHILUS HYDROTHERMALIS MEDIUM

KH_2PO_4	0.30	g
K_2HPO_4	0.30	g
NH_4Cl	0.50	g
KCl	0.10	g
NaCl	5.00	g
$MgCl_2$ x 6 H_2O	0.50	g
$CaCl_2$ x 2 H_2O	0.10	g
Yeast extract (OXOID)	5.00	g
Trypticase peptone (BD BBL)	5.00	g
Modified Wolin's mineral solution	10.00	ml
Na_2CO_3	3.00	g
Na-crotonate solution (1 M)	10.00	ml
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate, crotonate and sulfide), adjust pH to 8.5 and sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add crotonate and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. The crotonate solution should be sterilized by filtration. Adjust pH of the complete medium to 9.0, 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.

Na-crotonate solution (1 M) (from medium 870)

Crotonic acid (ALDRICH 113018)	86.00	g
NaOH (10 N)	100.00	ml
Distilled water	900.00	ml

Dissolve crotonic acid in 800 ml distilled water, add around 100 ml of 10 N NaOH and adjust pH to around 7. Then add water to reach a volume of 1000 ml. Sterilize by filtration under 100% N_2 gas atmosphere.