171: THERMOANAEROBACTER KIVUI MEDIUM

K_2HPO_4	0.22	g
KH_2PO_4	0.22	g
NaH_2PO_4 x H_2O	4.50	g
Na_2HPO_4 x 12 H_2O	6.10	g
NH_4Cl	0.31	g
$(NH_4)_2SO_4$	0.22	g
NaCl	0.45	g
$MgSO_4$ x 7 H_2O	0.09	g
$CaCl_2$ x 2 H_2O (0.1% w/v)	6.00	ml
$FeSO_4$ x 7 H_2O solution (0.1% w/v)	2.00	ml
Modified Wolin's mineral solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
L-Cysteine HCl x H_2O	0.50	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients except cysteine and sulfide, sparge medium with 80% H_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic, then distribute under same gas atmosphere into Hungate-type tubes or serum vials to 30% of their volume and autoclave. Add cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas atmosphere. Adjust pH of complete medium to 6.5.

2. After inoculation pressurize cultivation vessels to one bar overpressure with 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.

$FeSO_4$ x 7 H_2O solution (0.1% w/v) (from medium 119)

$FeSO_4$ x 7 H_2O	1.00	g
H_2SO_4 (0.1 N)	1000.00	ml

The ferrous sulfate solution is not stable and should be freshly prepared.