901a: THERMODESULFOBIUM ACIDIPHILUM MEDIUM

NH_4Cl	0.33	g
KCl	0.33	g
KH_2PO_4	0.33	g
$MgCl_2$ x 6 H_2O	0.33	g
$CaCl_2$ x 2 H_2O	0.33	g
Na_2SO_4	2.80	g
Trace element solution SL-10	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Yeast extract	3.00	g
Wolin's vitamin solution (10x)	1.00	ml
Neutralized sulfide solution 3% (w/v)	15.00	ml
Distilled water	1000.00	ml

Dissolve ingredients except yeast extract, vitamins and sulfide. Adjust pH to 4.5 with H_2SO_4 and sparge medium with 100% CO_2 gas for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add yeast extract, vitamins (sterilized by filtration) and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. The sulfide stock solution (3% w/v) should be neutralized before use (see medium 28). The pH of the complete medium should be at 4.5.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg

Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml

Neutralized sulfide solution 3% (w/v) (from medium 28)

Na_2S x 9 H_2O	3.00	g
Distilled water	100.00	ml

The sulfide solution is prepared in a 250 ml screw-capped bottle with a butyl rubber septum and a magnetic stirrer. The solution is bubbled with nitrogen gas, closed and autoclaved for 15 min. at 121°C. After cooling to room temperature the pH is adjusted to about 7.0 by adding of sterile 2 M H_2SO_4 drop-wise with a syringe without opening the bottle.