## **Microorganisms**



### 827: THERMOANAEROBACTER SULFUROPHILUS MEDIUM

NH <sub>4</sub> Cl	0.33	g
KCI	0.33	g
$KH_2PO_4$	0.33	g
$MgCl_2 \times 6 H_2O$	0.33	g
$CaCl_2 \times 2 H_2O$	0.33	g
Trace element solution SL-10	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Sulfur (powdered)	10.00	g
$Na_2CO_3$	1.00	g
Yeast extract	0.50	g
D-Glucose	5.00	g
Wolin's vitamin solution (10x)	1.00	ml
$Na_2S \times 9 H_2O$	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients except sulfur, carbonate, yeast extract, glucose, vitamins, and sulfide, then sparge medium with  $80\%~N_2$  and  $20\%~CO_2$  gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials containing already the appropriate amount of sulfur. Heat the medium vials to  $90\text{-}100^{\circ}\text{C}$  for 1 hour on each of 3 successive days to sterilize the medium. Add yeast extract, glucose, vitamins (sterilized by filtration), and sulfide from sterile anoxic stock solutions prepared under  $100\%~N_2$  gas and carbonate from a sterile anoxic stock solution prepared under  $80\%~N_2$  and  $20\%~CO_2$  gas mixture. Prior to use check pH of complete medium and adjust to 6.8 - 7.0, if necessary.

For <u>DSM 16547</u>: Supplement medium with 3.00 g/l Trypticase peptone.

### Trace element solution SL-10 (from medium 320)

HCI (25%)	10.00	ml
FeCl <sub>2</sub> x 4 H <sub>2</sub> O	1.50	g
ZnCl <sub>2</sub>	70.00	mg
MnCl <sub>2</sub> x 4 H <sub>2</sub> O	100.00	mg
H <sub>3</sub> BO <sub>3</sub>	6.00	mg
CoCl <sub>2</sub> x 6 H <sub>2</sub> O	190.00	mg
CuCl <sub>2</sub> x 2 H <sub>2</sub> O	2.00	mg
$NiCl_2 \times 6 H_2O$	24.00	mg
$Na_2MoO_4 \times 2 H_2O$	36.00	mg
Distilled water	990.00	ml

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

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

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCI	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B <sub>12</sub>	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml