Microorganisms



203. METHANOTHERMUS MEDIUM

Mineral solution 2 (see below) 37.5 mlNiCl2 x 6 H2O solution (0.1% w/v)1.0 mlFeSO4 x 7 H2O solution (0.1% w/v in 0.1 N H2SO4)2.0 mlTrace element solution (see medium 141)10.0 mlNa2SO43.4 gNa-resazurin solution (0.1% w/v)0.5 mlNa2CO31.0 gYeast extract (OXOID)2.0 gTrypticase peptone (BD BBL)2.0 gVitamin solution (see medium 141)10.0 mlNa2S x 9 H2O0.5 gL-Cysteine-HCI x H2O0.5 g	Mineral solution 1 (see below)	37.5	ml
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	Mineral solution 2 (see below)	37.5	ml
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	$NiCl_2 \times 6 H_2O$ solution (0.1% w/v)	1.0	ml
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	$FeSO_4 \times 7 H_2O$ solution (0.1% w/v in 0.1 N H_2SO_4)	2.0	ml
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	Trace element solution (see medium 141)	10.0	ml
$\begin{array}{cccc} \text{Na}_2\text{CO}_3 & 1.0 & \text{g} \\ \text{Yeast extract (OXOID)} & 2.0 & \text{g} \\ \text{Trypticase peptone (BD BBL)} & 2.0 & \text{g} \\ \text{Vitamin solution (see medium 141)} & 10.0 & \text{ml} \\ \text{Na}_2\text{S} \text{ x 9 H}_2\text{O} & 0.5 & \text{g} \\ \text{L-Cysteine-HCl x H}_2\text{O} & 0.5 & \text{g} \\ \end{array}$	Na_2SO_4	3.4	g
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	Na-resazurin solution (0.1% w/v)	0.5	ml
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Na_2CO_3	1.0	g
$\begin{array}{ccc} \text{Vitamin solution (see medium 141)} & 10.0 & \text{ml} \\ \text{Na}_2\text{S} \text{ x 9 H}_2\text{O} & 0.5 & \text{g} \\ \text{L-Cysteine-HCl x H}_2\text{O} & 0.5 & \text{g} \\ \end{array}$	Yeast extract (OXOID)	2.0	g
$Na_2S \times 9 H_2O$ 0.5 g L-Cysteine-HCl $\times H_2O$ 0.5 g	Trypticase peptone (BD BBL)	2.0	g
L-Cysteine-HCl x H_2O 0.5 g	Vitamin solution (see medium 141)	10.0	ml
-	$Na_2S \times 9 H_2O$	0.5	g
Distilled water 900 0 ml	L-Cysteine-HCl x H₂O	0.5	g
Distilled water 900.0 IIII	Distilled water	900.0	ml

Dissolve ingredients except carbonate, yeast extract, Trypticase peptone, vitamins, sulfide and cysteine, then adjust pH to 6.0 with sulfuric acid. Sparge medium with 80% $\rm H_2$ and 20% $\rm CO_2$ gas mixture for 30 – 45 min to make it anoxic. Dispense medium under same gas atmosphere into serum bottles, seal, and autoclave. Thereafter, add by injection yeast extract, Trypticase peptone, vitamins (sterilized by filtration), sulfide and cysteine from sterile anoxic stock solutions prepared under 100% $\rm N_2$ gas atmosphere and carbonate from a sterile anoxic stock solution prepared under 80% $\rm N_2$ and 20% $\rm CO_2$ gas mixture. Adjust pH of complete medium to 6.5. After inoculation pressurize bottles to 2 bar overpressure with sterile 80% $\rm H_2$ and 20% $\rm CO_2$ gas mixture.

Mineral sol	ulion	1:
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K ₂ HPO ₄	6.0	g
Distilled water	1000.0	ml
Mineral solution 2:		
Willieral Solution 2.		
KH ₂ PO ₄	6.0	g
$(NH_4)_2SO_4$	6.0	g
NaCl	12.0	g
$MgSO_4 \times 7 H_2O$	2.4	g
CaCl ₂ x 2 H ₂ O	1.6	g
Distilled water	1000.0	ml

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For <u>DSM</u> <u>3496</u> omit yeast extract and Trypticase peptone from the medium.