

1412. METHYLOTHERMUS MEDIUM

Mineral Salts Solution for strain HTM55:

NaCl	3.00	g
KH ₂ PO ₄	14.00	mg
CaCl x 2 H ₂ O	80.00	mg
MgSO ₄ x 7 H ₂ O	0.34	g
MgCl ₂ x 6 H ₂ O	0.42	g
KCl	33.00	g
NH ₄ Cl	0.25	g
NaNO ₃	0.25	g
Na ₂ SiO ₃ x 9 H ₂ O	0.20	mg
Fe ₂ (SO ₄) ₃ x n H ₂ O	20.00	mg
Na ₂ SeO ₃ 5 H ₂ O	0.50	mg
NiCl ₂ x 6 H ₂ O	0.50	mg
Trace Element Solution (see below)	3.00	ml
Vitamin Solution (see below)	1.00	ml
Distilled Water	1000.00	ml

Mineral Salts Solution for strain IT-4 and IT-9:

NaCl	30.00	g
KH ₂ PO ₄	140.00	mg
CaCl x 2 H ₂ O	800.00	mg
MgSO ₄ x 7 H ₂ O	3.40	g
MgCl ₂ x 6 H ₂ O	4.18	g
KCl	0.33	g
NH ₄ Cl	0.25	g
NaNO ₃	0.25	g
Na ₂ SiO ₃ x 9 H ₂ O	0.20	mg
Fe ₂ (SO ₄) ₃ x n H ₂ O	20.00	mg
Na ₂ SeO ₃ 5 H ₂ O	0.50	mg
NiCl ₂ x 6 H ₂ O	0.50	mg
Trace Element Solution (see below)	3.00	ml
Vitamin Solution (see below)	1.00	ml
Distilled Water	1000.00	ml

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Trace Element Solution:

Nitrilotriacetic Acid	1.50	g
MnSO ₄ x 2 H ₂ O	0.50	g
CoSO ₄ x 7 H ₂ O	0.18	g
ZnSO ₄ x 7 H ₂ O	0.18	g
CuSO ₄ x 5 H ₂ O	0.01	g
KAl(SO ₄) x 12 H ₂ O	0.02	g
H ₃ BO ₄	0.01	g
Na ₂ MoO ₄ x 2 H ₂ O	0.01	g
Distilled Water	1000.00	ml

Vitamin Solution:

Biotin	2.0	mg
Folic Acid	2.0	mg
Pyridoxine-HCl	10.0	mg
Thiamine-HCl	5.0	mg
Riboflavin	5.0	mg
Nicotinic Acid	5.0	mg
D-Ca-Pantothenate	5.0	mg
Vitamin B ₁₂	0.1	mg
p-Aminobenzoic Acid	5.0	mg
Lipoic Acid	5.0	mg
Distilled Water	1000.000	ml

Prepare the liquid medium. Adjust to pH 6.2 with HCl or NaOH. Dispense the medium into serum bottles under gassing with N₂:CO₂ (80:20 v/v) for one minute. 20% methane and 5% air is added to the gas phase

Sterilize by autoclaving. Before inoculation add from sterile stock solutions:

Vitamin solution	1.0	ml/l
NaHCO ₃ (5% w/v)	50.0	ml/l
CuSO ₄ x 5 H ₂ O (0.1 M)	10.0	ml/l

For DSM 109509 use Medium 1412 for strain IT4:

Before inoculation add from sterile stock solutions:

Vitamin solution	1.0	ml/l
NaHCO ₃ (10% w/v)	1.0	ml/l
CuSO ₄ x 5 H ₂ O (1 mM)	1.0	ml/l