

120: METHANOSARCINA MEDIUM

This recipe contains strain-specific modifications for *Methanosarcina thermophila* DSM 1825 *

Final pH: * 6.5 - 6.8

Final volume: 1024 ml

K ₂ HPO ₄	0.35	g
KH ₂ PO ₄	0.23	g
NH ₄ Cl	0.50	g
MgSO ₄ x 7 H ₂ O	0.50	g
CaCl ₂ x 2 H ₂ O	0.25	g
NaCl	2.25	g
FeSO₄ x 7 H₂O solution (0.1% w/v)	2.00	ml
Trace element solution SL-10	1.00	ml
Yeast extract (OXOID)	2.00	g
Casitone (BD BBL)	2.00	g
Na-acetate	2.50	g
Sodium resazurin (0.1% w/v)	0.50	ml
NaHCO ₃	2.00	g
Methanol (50% v/v)	20.00	ml
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H ₂ O	0.30	g
Na ₂ S x 9 H ₂ O	0.30	g
Clarified rumen fluid	50.00	ml
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, vitamins, methanol, cysteine and sulfide) and sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Then add and dissolve bicarbonate, adjust pH to 6.5 and dispense medium under 80% N₂ and 20% CO₂ gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. Methanol (50% v/v stock solution) and the reducing agents are each autoclaved separately under 100% N₂ gas atmosphere as concentrated solutions in tightly closed tubes. Vitamins are prepared under 100% N₂ gas atmosphere and sterilized by filtration. Appropriate volumes of the stock solutions are injected into the sterile medium with hypodermic syringes. Adjust pH of the complete medium to 6.8 - 7.0, if necessary.

* Supplement medium with 5% (v/v) clarified rumen fluid (see medium 1310) and adjust pH to 6.5 - 6.8.

FeSO₄ x 7 H₂O solution (0.1% w/v) (from medium 119)

FeSO ₄ x 7 H ₂ O	1.00	g
H ₂ SO ₄ (0.1 N)	1000.00	ml

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

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
FeCl ₂ x 4 H ₂ O	1.50	g
ZnCl ₂	70.00	mg
MnCl ₂ x 4 H ₂ O	100.00	mg
H ₃ BO ₃	6.00	mg
CoCl ₂ x 6 H ₂ O	190.00	mg
CuCl ₂ x 2 H ₂ O	2.00	mg
NiCl ₂ x 6 H ₂ O	24.00	mg
Na ₂ MoO ₄ x 2 H ₂ O	36.00	mg
Distilled water	990.00	ml

First dissolve FeCl₂ 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

Clarified rumen fluid* (from medium 1310)

Rumen fluid from cow or sheep (obtained from fistulated animals or abattoir refuse) is filtered through muslin, autoclaved at 121°C for 15 min and then centrifuged at 27,000 g for 20 min. The supernatant is made anoxic by sparging with 100% N₂ gas for 15 min, dispensed under same gas atmosphere into anoxic serum vials to 30% of volume and then stored frozen at -20°C.