

734: METHANOBREVIBACTER CURVATUS MEDIUM

This recipe contains strain-specific modifications for *Methanolapillus africanus* DSM 115569 *

Final pH: 7.2

Final volume: 1003 ml

Clarified rumen fluid	400.00	ml
NaCl	1.00	g
KCl	0.50	g
MgCl ₂ x 6 H ₂ O	0.40	g
CaCl ₂ x 2 H ₂ O	0.10	g
NH ₄ Cl	0.30	g
KH ₂ PO ₄	0.20	g
Na ₂ SO ₄	0.15	g
Casamino acids (BD Bacto)	0.50	g
Yeast extract (OXOID)	0.50	g
Nutrient broth (BD Difco)	2.00	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na ₂ CO ₃	1.00	g
MOPS (SIGMA)	2.10	g
Seven vitamins solution	1.00	ml
DL-Dithiothreitol (DTT)	0.16	g
Methanol (50% v/v)	20.00	ml
Distilled water	600.00	ml

Dissolve ingredients (except carbonate, MOPS buffer, vitamins and DTT), bring medium to the boil, then cool to room temperature under 80% H₂ and 20% CO₂ gas mixture. Dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add MOPS buffer adjusted to pH 7.2, vitamins and DTT from anoxic stock solutions prepared under 100% N₂ gas and sterilized by filtration and carbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. Adjust pH of complete medium to 7.2, if necessary.

* Supplement medium after autoclaving with 20 ml/l methanol (50% v/v) added from a sterile anoxic stock solution prepared under 100% N₂.

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

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stored frozen at -20°C.

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.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na ₂ SeO ₃ x 5 H ₂ O	3.00	mg
Na ₂ WO ₄ x 2 H ₂ O	4.00	mg
Distilled water	1000.00	ml

Seven vitamins solution (from medium 503)

Vitamin B ₁₂	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H ₂ O	200.00	mg
Distilled water	1000.00	ml