119: METHANOBACTERIUM MEDIUM

This recipe contains strain-specific modifications for *Acetitomaculum ruminis* DSM 5522 *

Final pH: 6.8 - 7.0
Final volume: 1003 ml

KH_2PO_4	0.50	g
$MgSO_4$ x 7 H_2O	0.40	g
NaCl	0.40	g
NH_4Cl	0.40	g
$CaCl_2$ x 2 H_2O	0.05	g
Trace element solution SL-10	1.00	ml
Yeast extract (OXOID)	1.00	g
Na-acetate	1.00	g
Na-formate	2.00	g
$FeSO_4$ x 7 H_2O solution (0.1% w/v)	2.00	ml
Sludge fluid	50.00	ml
Fatty acid mixture	20.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	4.00	g
L-Cysteine HCl x H_2O	0.50	g
Na_2S x 9 H_2O	0.50	g
D-Glucose	5.00	g
Distilled water	930.00	ml

1. Dissolve ingredients except bicarbonate, cysteine and sulfide. Sparge medium with 80% H_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Add and dissolve bicarbonate, adjust pH to 6.5 and dispense medium under 80% H_2 and 20% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. Add cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. Prior to use check pH of complete medium and adjust to 6.8 - 7.0, if necessary.

2. Note: After growth has started and the culture is becoming turbid add sterile 80% H_2 and 20% CO_2 gas mixture to 0.5 - 1 bar overpressure.

* Supplement medium with 5.0 g/l D-glucose added after autoclaving from a sterile anoxic stock solution.

Sludge fluid (from medium 119)

Yeast extract	4.00	g
Sludge	1000.00	ml

Add 0.4% yeast extract to sludge from an anaerobic digester, and after gassing with

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nitrogen gas for a few minutes incubate it at 37°C for 24 hours. Then centrifuge the sludge at 13000 g and autoclave the resulting, clear supernatant in screw-capped vessels under nitrogen gas. The sludge fluid can be stored at 8-12°C in the dark.

Fatty acid mixture (from medium 119)

Isobutyric acid	23.00	ml
DL-2-Methylbutyric acid	27.00	ml
Valeric acid	27.00	ml
Isovaleric acid	27.00	ml
Distilled water	896.00	ml

Adjust pH to 7.5 with concentrated NaOH.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ x 4 H_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 2 H_2O	36.00	mg
Distilled water	990.00	ml

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

$FeSO_4$ x 7 H_2O solution (0.1% w/v) (from medium 119)

$FeSO_4$ x 7 H_2O	1.00	g
H_2SO_4 (0.1 N)	1000.00	ml

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

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