734: METHANOBREVIBACTER CURVATUS MEDIUM

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_2O	0.40	g
$CaCl_2$ x 2 H_2O	0.10	g
NH_4Cl	0.30	g
KH_2PO_4	0.20	g
Na_2SO_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_2CO_3	1.00	g
MOPS (SIGMA)	2.10	g
Seven vitamins solution	1.00	ml
DL-Dithiothreitol (DTT)	0.16	g
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% H_2 and 20% CO_2 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% N_2 gas and sterilized by filtration and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Adjust pH of complete medium to 7.2, if necessary.

For DSM 25720, DSM 115569: Supplement medium after autoclaving with 20 ml/l methanol (50% v/v) added from a sterile anoxic stock solution prepared under 100% N_2.

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% N_2 gas for 15 min, dispensed under same gas atmosphere into anoxic serum vials to 30% of volume and then stored frozen at -20°C.

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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.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	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_2O	200.00	mg
Distilled water	1000.00	ml

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