

322: METHANOSPHERA MEDIUM

Clarified rumen fluid	100.00	ml
Trypticase peptone (BD BBL)	2.00	g
Yeast extract	2.00	g
Na-acetate	0.50	g
Na-formate	0.50	g
Modified Wolin's mineral solution	10.00	ml
Na ₂ SeO ₄ (0.1% w/v)	1.90	ml
NiCl ₂ × 6 H ₂ O (0.1% w/v)	0.70	ml
FeSO₄ × 7 H₂O solution (0.1% w/v)	3.00	ml
K ₂ HPO ₄	0.60	g
KH ₂ PO ₄	2.80	g
(NH ₄) ₂ SO ₄	0.30	g
NH ₄ Cl	1.00	g
NaCl	0.60	g
MgSO ₄ × 7 H ₂ O	0.15	g
CaCl ₂ × 2 H ₂ O	0.08	g
Sodium resazurin (0.1% w/v)	0.50	ml
NaHCO ₃	4.00	g
Methanol (50% v/v)	10.00	ml
Wolin's vitamin solution (10x)	2.00	ml
DL-Dithiothreitol	0.50	g
Distilled water	900.00	ml

1. Dissolve ingredients except bicarbonate, methanol, and dithiothreitol (DTT), then sparge medium with 80% H₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Add and dissolve bicarbonate, adjust pH of medium to 6.8 - 7.0, then dispense medium under 80% H₂ and 20% CO₂ gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. Add methanol (50% v/v solution), vitamins, and DTT from sterile anoxic stock solutions prepared under 100% N₂ gas. Vitamins and DTT should be sterilized by filtration. Prior to use check pH of complete medium and adjust to 6.7 - 6.9, if necessary.
2. After inoculation add sterile 80% H₂ and 20% CO₂ gas mixture to 1 bar overpressure.

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.

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Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
MgSO ₄ x 7 H ₂ O	3.00	g
MnSO ₄ x H ₂ O	0.50	g
NaCl	1.00	g
FeSO ₄ x 7 H ₂ O	0.10	g
CoSO ₄ x 7 H ₂ O	0.18	g
CaCl ₂ x 2 H ₂ O	0.10	g
ZnSO ₄ x 7 H ₂ O	0.18	g
CuSO ₄ x 5 H ₂ O	0.01	g
AlK(SO ₄) ₂ x 12 H ₂ O	0.02	g
H ₃ BO ₃	0.01	g
Na ₂ MoO ₄ x 2 H ₂ O	0.01	g
NiCl ₂ x 6 H ₂ O	0.03	g
Na ₂ SeO ₃ x 5 H ₂ O	0.30	mg
Na ₂ WO ₄ x 2 H ₂ O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

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

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

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