

1523: MODIFIED METHANOBACTERIUM MEDIUM

Final pH: 6.8 - 7.0

Final volume: 1002 ml

KH ₂ PO ₄	0.50	g
MgSO ₄ x 7 H ₂ O	0.40	g
NaCl	0.40	g
NH ₄ Cl	0.40	g
CaCl ₂ x 2 H ₂ O	0.05	g
Trace element solution SL-10	1.00	ml
Brain heart infusion (BD Bacto)	6.00	g
Proteose peptone (BD Difco)	6.00	g
Yeast extract (OXOID)	2.00	g
Na-acetate	1.00	g
Na-formate	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
NaHCO ₃	4.00	g
Seven vitamins solution	1.00	ml
L-Cysteine HCl x H ₂ O	0.50	g
Na ₂ S x 9 H ₂ O	0.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients except bicarbonate, vitamins, cysteine, and sulfide, then sparge medium with 80% H₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Add and dissolve bicarbonate and adjust pH to 6.5. 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 vitamins, cysteine, and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas. The vitamin solution should be sterilized by filtration. Before use, check the complete medium's pH and adjust it to 6.8 - 7.0, if necessary.

2. After growth has started and the culture is becoming turbid add sterile 80% H₂ and 20% CO₂ gas mixture to 0.5 - 1 bar overpressure.

For [DSM 1093](#), [DSM 1125](#), [DSM 11977](#), [DSM 11978](#), [DSM 16643](#): Supplement medium after autoclaving with 0.50 g/l coenzyme M (2-mercaptoethanesulfonic acid) and 0.30 g/l DTT (DL-Dithiothreitol) added from filter-sterilized anoxic stock solutions prepared under N₂. Omit sulfide and cysteine from the medium.

For [DSM 1535](#): Adjust pH of complete medium to 7.6.

For [DSM 2640](#), [DSM 2702](#), [DSM 3823](#), [DSM 4140](#), [DSM 4179](#), [DSM 4273](#), [DSM 4274](#), [DSM 10196](#): Increase amount of Na-acetate to 4.00 g/l.

For [DSM 15163](#): Adjust pH of complete medium to 6.0.

For [DSM 16632](#): Supplement medium after autoclaving with 50 ml/l clarified rumen fluid

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and 0.3 g/l DTT (DL-dithiothreitol). Omit $\text{Na}_2\text{S} \times 9 \text{H}_2\text{O}$.

For [DSM 115763](#), [DSM 115764](#), [DSM 115765](#): Supplement medium after autoclaving with 0.30 g/l DTT (DL-dithiothreitol), 10.00 ml/l methanol (50 % v/v), and 0.50 g/l coenzyme M (2-mercaptoethanesulfonic acid) added from filter-sterilized anoxic stock solutions prepared under N_2 . Omit $\text{Na}_2\text{S} \times 9 \text{H}_2\text{O}$ and L-cysteine HCl $\times \text{H}_2\text{O}$.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$\text{FeCl}_2 \times 4 \text{H}_2\text{O}$	1.50	g
ZnCl_2	70.00	mg
$\text{MnCl}_2 \times 4 \text{H}_2\text{O}$	100.00	mg
H_3BO_3	6.00	mg
$\text{CoCl}_2 \times 6 \text{H}_2\text{O}$	190.00	mg
$\text{CuCl}_2 \times 2 \text{H}_2\text{O}$	2.00	mg
$\text{NiCl}_2 \times 6 \text{H}_2\text{O}$	24.00	mg
$\text{Na}_2\text{MoO}_4 \times 2 \text{H}_2\text{O}$	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.

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 $\times 2 \text{H}_2\text{O}$	200.00	mg
Distilled water	1000.00	ml