

1523. MODIFIED METHANOBACTERIUM MEDIUM

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 (see medium 320)	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
Na-resazurin solution (0.1% w/v)	0.50	ml
NaHCO ₃	4.00	g
Vitamins solution (see medium 503)	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

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, then dispense medium under 80% H₂ and 20% CO₂ gas atmosphere into anoxic Hungate-type tubes or serum vials 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. Prior to use check pH of complete medium and adjust to 6.8 - 7.0, if necessary. 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](#) supplement medium after autoclaving with 0.50 g/l coenzyme M (2-mercaptoethanesulfonic acid) added from a filter-sterilized anoxic stock solution prepared under N₂.

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

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