1523. MODIFIED METHANOBACTERIUM MEDIUM

KH₂PO₄ 0.50 g
MgSO₄ x 7 H₂O 0.40 g
NaCl 0.40 g
NH₄Cl 0.40 g
CaCl₂ x 2 H₂O 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
NaHCO₃ 4.00 g
Vitamins solution (see medium 503) 1.00 ml
L-Cysteine-HCl x H₂O 0.50 g
Na₂S x 9 H₂O 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.