

1637. METHANOMASSILIICOCCUS LUMINYENSIS MEDIUM

Trypticase peptone (BD BACTO)	16.0	g
Yeast extract (OXOID)	7.0	g
NaCl	5.0	g
L-Cysteine-HCl x H ₂ O	0.5	g
NaHCO ₃	0.5	g
Haemin solution (0.05% w/v, see medium 78)	5.0	ml
Vitamin K3 solution (0.005% w/v, see medium 78)	5.0	ml
Na-resazurin solution (0.1% w/v)	0.5	ml
Methanol	1.5	ml
DL-Dithiothreitol	1.0	g
Distilled water	1000.0	ml

Dissolve ingredients except methanol and dithiothreitol. Adjust pH to 7.5 with 1 N NaOH and sparge medium with 100% N₂ gas for 30 – 45 min to make it anoxic. Distribute medium under 100% N₂ gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. After sterilization add methanol from a sterile anoxic stock solution autoclaved under 100% N₂ gas atmosphere and dithiothreitol from an anoxic stock solution sterilized by filtration. Prior to use check pH of complete medium and adjust to 7.6 – 7.8, if necessary. After inoculation, add sterile 80% H₂ and 20% CO₂ gas mixture to 0.5 bar overpressure. After growth becomes visible overpressure of 80% H₂ and 20% CO₂ gas mixture can be increased to 1 bar.

Note: Use 10% (v/v) as inoculum.