

1318. METHANOCELLA CONRADII MEDIUM

MgCl ₂ x 6 H ₂ O	0.40	g
CaCl ₂ x 2 H ₂ O	0.10	g
NH ₄ Cl	0.10	g
KH ₂ PO ₄	0.20	g
KCl	0.50	g
Yeast extract	0.20	g
Na-acetate	0.09	g
Trace element solution SL-10 (see medium 320)	1.00	ml
Selenite-tungstate solution (see medium 385)	1.00	ml
Na-resazurin solution (0.1% w/v)	0.50	ml
NaHCO ₃	2.50	g
Vitamin solution (see medium 141)	9.00	ml
Seven vitamins solution (see medium 503)	1.00	ml
Na ₂ S x 9 H ₂ O	0.20	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, vitamins and sulfide) and sparge medium with 80% H₂ and 20% CO₂ gas mixture for 30 – 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add vitamins (sterilized by filtration) and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. The pH of the complete medium should be 7.0. After inoculation pressurize vials to 1 bar overpressure with sterile 80% H₂ and 20% CO₂ gas mixture.