

511. METHANOPYRUS MEDIUM

NH ₄ Cl	0.25	g
K ₂ HPO ₄ x 3 H ₂ O	0.07	g
KH ₂ PO ₄	0.09	g
NaCl	11.80	g
MgSO ₄ x 7 H ₂ O	1.75	g
MgCl ₂ x 6 H ₂ O	4.50	g
Na ₂ SO ₄	0.81	g
CaCl ₂ x 2 H ₂ O	0.78	g
KCl	0.30	g
Trace element solution (see medium 141)	10.00	ml
(NH ₄) ₂ Fe(SO ₄) ₂ x 6 H ₂ O solution (0.1% w/v)	2.00	ml
(NH ₄) ₂ Ni(SO ₄) ₂ solution (0.1% w/v)	2.00	ml
Na ₂ WO ₄ x 2 H ₂ O solution (0.1% w/v)	2.00	ml
Marine trace elements (see below)	10.00	ml
Na-resazurin solution (0.1% w/v)	0.50	ml
NaHCO ₃	1.00	g
Vitamins solution (see medium 141)	10.00	ml
Na ₂ S x 9 H ₂ O	0.50	g
Distilled water	970.00	ml

Dissolve ingredients except bicarbonate, vitamins and sulfide, then sparge medium with 80% H₂ and 20% CO₂ gas mixture for 30 – 45 min to make it anoxic, then dispense under same gas atmosphere into serum vials and autoclave. Add vitamins 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. Vitamins should be sterilized by filtration. Adjust pH of the complete medium to 6.5, if necessary. After inoculation pressurize vessels to 2 bar overpressure with sterile 80% H₂ and 20% CO₂ gas mixture.

Marine trace elements solution:

NaBr	4.00	g
SrCl ₂ x 6 H ₂ O	1.80	g
H ₃ BO ₃	1.30	g
KI	1.25	mg
Na-silicate	100.00	mg
NaF	60.00	mg
KNO ₃	40.00	mg
Na ₂ HPO ₄ x 3 H ₂ O	0.25	mg
Distilled water	1000.00	ml