

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
Modified Wolin's mineral solution	10.00	ml
Fe(NH ₄) ₂ (SO ₄) ₂ x 6 H ₂ O (0.1% w/v)	2.00	ml
(NH ₄) ₂ Ni(SO ₄) ₂ (0.1% w/v)	2.00	ml
Na ₂ WO ₄ x 2 H ₂ O (0.1% w/v)	2.00	ml
Marine trace elements solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
NaHCO ₃	1.00	g
Wolin's vitamin solution (10x)	1.00	ml
Na ₂ S x 9 H ₂ O	0.50	g
Distilled water	980.00	ml

1. 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 to 30% of their volume 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.

2. 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

Modified Wolin's mineral solution (from medium 141)

Nitrolotriacetic acid	1.50	g
MgSO ₄ x 7 H ₂ O	3.00	g
MnSO ₄ x H ₂ O	0.50	g
NaCl	1.00	g
FeSO ₄ x 7 H ₂ O	0.10	g
CoSO ₄ x 7 H ₂ O	0.18	g
CaCl ₂ x 2 H ₂ O	0.10	g
ZnSO ₄ x 7 H ₂ O	0.18	g
CuSO ₄ x 5 H ₂ O	0.01	g
AlK(SO ₄) ₂ x 12 H ₂ O	0.02	g
H ₃ BO ₃	0.01	g
Na ₂ MoO ₄ x 2 H ₂ O	0.01	g
NiCl ₂ x 6 H ₂ O	0.03	g
Na ₂ SeO ₃ x 5 H ₂ O	0.30	mg
Na ₂ WO ₄ x 2 H ₂ O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrolotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B ₁₂	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml