

1280: METHANOREGULA (PEAT) MEDIUM

Solution A	954.00	ml
Solution B	10.00	ml
Solution C	13.00	ml
Solution D	10.00	ml
Solution E	10.00	ml
Solution F	10.00	ml

1. Dissolve ingredients of solution A, adjust pH to 5.0 with 0.1 N HCl and sparge with 80% H₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Dispense solution A under same gas atmosphere into anoxic Hungate-type tubes or serum vials (e.g., 20 ml medium in 50 ml bottles) and autoclave. Solutions B - F are prepared under 100% N₂ gas atmosphere and sterilized by filtration. Prior to inoculation complete the medium by adding appropriate amounts of solutions B to F to the sterile solution A in the sequence as indicated.

2. Adjust pH of the complete medium to 5.1 with a sterile anoxic solution of 1 N HCl. After inoculation pressurize vials to 1 bar overpressure with sterile 80% H₂ and 20% CO₂ gas mixture.

3. Note: Use 10-20% (v/v) as inoculum.

Solution A

NH ₄ Cl	27.00	mg
KH ₂ PO ₄	14.00	mg
KCl (0.1% w/v)	2.00	ml
Na-acetate	17.00	mg
Trace elements solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	950.00	ml

Solution B

Homo-PIPES (SIGMA)	1.58	g
NaOH (pellets)	0.18	g
Distilled water	10.00	ml

Adjust pH of buffer to 5.5.

Solution C

NaOH (0.38 M)	7.20	ml
Nitrilotriacetate (0.5 M, neutralized with 10 N NaOH)	4.80	ml
TiCl ₃ (15% w/v in HCl, RIEDEL-de HAEN)	0.55	ml

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Solution D

Yeast extract	0.20	g
Distilled water	10.00	ml

Solution E

2-Mercaptoethanesulfonate (coenzyme M)	0.07	g
Distilled water	10.00	ml

Solution F

Wolin's vitamin solution (10x)	10.00	ml
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Trace elements solution

HCl (25%)	10.00	ml
FeCl ₂ x 4 H ₂ O	1.34	g
CoCl ₂ x 6 H ₂ O	24.00	mg
ZnCl ₂	75.00	mg
H ₃ BO ₃	19.00	mg
NiCl ₂ x 6 H ₂ O	24.00	mg
Na ₂ MoO ₄ x 2 H ₂ O	24.00	mg
MnSO ₄ x 4 H ₂ O	26.00	mg
MgSO ₄ x 7 H ₂ O	1.56	g
CaCl ₂ x 2 H ₂ O	2.34	g
CuSO ₄ x 5 H ₂ O	9.00	mg
AlK(SO ₄) ₂ x 12 H ₂ O	3.45	g
Distilled water	1000.00	ml

First dissolve ferrous chloride in the HCl, then dilute in water and dissolve the other salts. Finally make up to 1000 ml.

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