

1280. METHANOREGULA (PEAT) MEDIUM

Solution A:

NH ₄ Cl	27.00 mg
KH ₂ PO ₄	14.00 mg
KCl solution (0.1% w/v)	2.00 ml
Na-acetate	17.00 mg
Trace elements solution (see below)	1.00 ml
Na-resazurin solution (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
Nitritotriacetate solution (0.5 M, neutralized with 10 N NaOH)	4.80 ml
TiCl ₃ solution (15% w/v in HCl, RIEDEL-de HAEN)	0.55 ml

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:

Vitamins solution (see medium 141, but 10x conc.)	10.00 ml
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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.

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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.

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

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.