

## 1094. METHANOSPHERULA (PEAT) MEDIUM

### Solution A:

KCl solution (0.1% w/v)	1.50 ml
KH <sub>2</sub> PO <sub>4</sub> solution (0.1% w/v)	13.60 ml
NH <sub>4</sub> Cl solution (0.1% w/v)	26.80 ml
Trace element solution (see below)	1.00 ml
Distilled water	940.00 ml

### Solution B:

TRIS-HCl (1.0 M solution, pH 8.0)	7.20 ml
NaNTA (0.5 M solution)	4.80 ml
TiCl <sub>3</sub> (15% w/v solution in HCl; Riedel-de Haen)	0.55 ml

### Solution C:

2-(N-Morpholino)ethanesulfonic acid (MES)	1.95 g
NaOH	0.20 g
Distilled water	20.00 ml

Adjust to pH 7.5 with NaOH.

### Solution D:

Vitamin solution (see medium 141)	10.00 ml
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### Solution E:

2-Mercaptoethanesulfonic acid (Coenzyme M)	0.08 g
Distilled water	10.00 ml

### Solution F:

Na-acetate	0.41 g
Distilled water	10.00 ml

### Solution G:

Na <sub>2</sub> S x 9 H <sub>2</sub> O	9.60 mg
Distilled water	10.00 ml

Sparge *solution A* with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 – 45 min to make anoxic. Dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. *Solutions B, C, D* and *G* are prepared under 100% N<sub>2</sub> gas atmosphere and sterilized by filtration. *Solutions E* and *F* are prepared under 100% N<sub>2</sub> gas atmosphere and autoclaved.

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To complete the medium add appropriate amounts of *solutions B to G* to the sterile solution A in the sequence indicated and let medium equilibrate overnight. After inoculation pressurize vials to 1 bar overpressure with sterile 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. Adjust pH of complete medium to 5.7, if necessary.

*Note: The complete medium is not stable and can be stored only for a short time.*

*Trace element solution:*

Na <sub>2</sub> -EDTA	37.23	g
FeCl <sub>2</sub> x 4 H <sub>2</sub> O	1.34	g
AlK(SO <sub>4</sub> ) <sub>2</sub> x 12 H <sub>2</sub> O	3.45	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	2.34	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	1.56	g
CoCl <sub>2</sub> x 6 H <sub>2</sub> O	24.00	mg
ZnCl <sub>2</sub>	75.00	mg
H <sub>3</sub> BO <sub>3</sub>	19.00	mg
NiCl <sub>2</sub> x 6 H <sub>2</sub> O	24.00	mg
Na <sub>2</sub> Mo <sub>4</sub> x 2 H <sub>2</sub> O	24.00	mg
MnSO <sub>4</sub> x 4 H <sub>2</sub> O	26.00	mg
CuSO <sub>4</sub> x 5 H <sub>2</sub> O	9.00	mg
Distilled water	1000.00	ml

Dissolve EDTA in distilled water, adjust pH to 7 using 2 N NaOH and add ferrous chloride. After ferrous chloride has dissolved add other compounds.

For [DSM 25616](#) supplement medium with 0.02 g/l yeast extract added to the sterile medium from an anoxic stock solution sterilized by filtration. Adjust pH of complete medium to **6.2**. Use 5-10 vol% as inoculum and incubate while shaking at 100 – 200 rpm.

For [DSM 25820](#) supplement medium with 0.02 g/l yeast extract added to the sterile medium from an anoxic stock solution sterilized by filtration. Use 5-10 vol% as inoculum and incubate while shaking at 100 – 200 rpm.