

1094: METHANOSPHERULA (PEAT) MEDIUM

Solution A	943.00	ml
Solution B	13.00	ml
Solution C	20.00	ml
Solution D	1.00	ml
Solution E	10.00	ml
Solution F	10.00	ml
Solution G	10.00	ml

1. Sparge solution A with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make anoxic. Dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. Solutions B, C, D and G are prepared under 100% N₂ gas atmosphere and sterilized by filtration. Solutions E and F are prepared under 100% N₂ gas atmosphere and autoclaved. 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. Adjust pH of complete medium to 5.7, if necessary.
2. After inoculation pressurize vials to 1 bar overpressure with sterile 80% H₂ and 20% CO₂ gas mixture.
3. Note: The complete medium is not stable and can be stored only for a short time.

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.

Solution A

KCl (0.1% w/v)	1.50	ml
KH ₂ PO ₄ (0.1% w/v)	13.60	ml
NH ₄ Cl (0.1% w/v)	26.80	ml
Trace element solution	1.00	ml
Distilled water	900.00	ml

Solution B

Tris-HCl buffer (1.0 M solution, pH 8.0)	7.20	ml
Na ₃ -NTA x H ₂ O (0.5 M solution)	4.80	ml
TiCl ₃ (15% w/v solution in HCl; Riedel-de Haen)	0.55	ml

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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

Wolin's vitamin solution (10x)	1.00	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 ₂ S x 9 H ₂ O	9.60	mg
Distilled water	10.00	ml

Trace element solution

Na ₂ -EDTA	37.23	g
FeCl ₂ x 4 H ₂ O	1.34	g
AlK(SO ₄) ₂ x 12 H ₂ O	3.45	g
CaCl ₂ x 2 H ₂ O	2.34	g
MgSO ₄ x 7 H ₂ O	1.56	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 Mo ₄ x 2 H ₂ O	24.00	mg
MnSO ₄ x 4 H ₂ O	26.00	mg
CuSO ₄ x 5 H ₂ 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.

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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