

1610. METHYLOCEANIBACTER MEDIUM (NaCl-dANMS)**Solution 1** (50x salt stock):

MgSO ₄ x 6 H ₂ O	10.00	g
CaCl ₂ x 2 H ₂ O	1.50	g
Distilled water	1000.00	ml

Dissolve the ingredients listed above (in that order) in about 700 ml of distilled water, and then make up to 1 litre

Solution 2 (100 mM Fe EDTA):

Fe EDTA	36.05	g
Distilled water	1000.00	ml

Solution 3 (100 mM KH₂PO₄):

KH ₂ PO ₄	13.60	g
Distilled water	1000.00	ml

Solution 4 (10 mM CuSO₄ x 5 H₂O):

KH ₂ PO ₄	2.50	g
Distilled water	1000.00	ml

Solution 5 (30% NaCl):

NaCl	30.00	g
Distilled water	1000.00	ml

Solution 6 (100 mM NH₄Cl):

NH ₄ Cl	5.30	g
Distilled water	1000.00	ml

Solution 7 (100 mM KNO₃):

KNO ₃	10.10	g
Distilled water	1000.00	ml

Solution 8 (100 mM Hepes):

Hepes	23.80	g
Distilled water	1000.00	ml

Solution 9 (100µM lanthanides):

CeCl ₃ x 7H ₂ O	0.04	g
LaCl ₃ x 7H ₂ O	0.04	g
NdCl ₃ x 6H ₂ O	0.04	g
PrCl ₃ x H ₂ O	0.02	g
Distilled water	1000.00	ml

Trace element solution:

EDTA	0.500	g
FeSO ₄ x 7 H ₂ O	0.200	g
ZnSO ₄	0.010	g
MnCl ₂	0.003	g
H ₃ BO ₃	0.030	g
CoCl ₂ x 6 H ₂ O	0.020	g
CuCl ₂ x 5 H ₂ O	0.010	g
NiCl ₂ x 6 H ₂ O	0.020	g
Na ₂ MoO ₄ x 2 H ₂ O	0.002	g
Distilled water	1000.00	ml

May be stored at 4°C in the dark

Prepare the growth medium as follows:

Dilute 20 ml of solution 1 to 700ml with distilled water.

Add 100mL of solution 5, 50 mL of solution 8, 20mL of solution 6 and 7 and 10 mL of solution 3.

Add 1ml trace element solution.

Add 0.4 mL solution 2.

Add 0.1 mL solution 4.

Add 1.0 mL solution 9.

Disolve and bring to 1L.

Adjust pH to 7.8 with HCl or NaOH.

Autoclave medium.

After autoclaving add 10mL per liter of filter sterilized methanol.