

921. METHYLOSARCINA QUISQUILLARUM AND M. FIBRATA MEDIUM

Solution 1 (10x NMS salts):

KNO ₃	10.00	g
MgSO ₄ x 6 H ₂ O	10.00	g
CaCl ₂ x 2 H ₂ O	2.00	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 (Fe EDTA):

Fe EDTA	3.80	g
Distilled water	1000.00	ml

Solution 3 (Sodium molybdate):

Na ₂ MoO ₄ x 2 H ₂ O	0.26	g
Distilled water	1000.00	ml

Trace elements:

EDTA di sodium salt	25.00	mg
CuSO ₄ x 5 H ₂ O	100.00	mg
FeSO ₄ x 7 H ₂ O	50.00	mg
ZnSO ₄ x 7 H ₂ O	40.00	mg
H ₃ BO ₃	1.50	mg
CoCl ₂ x 6 H ₂ O	5.00	mg
MnCl ₂ x 4 H ₂ O	2.00	mg
NiCl ₂ x 6 H ₂ O	1.00	mg
Distilled water	100.00	ml

May be stored at 4°C in the dark

Phosphate buffer:

Na ₂ HPO ₄ x 12 H ₂ O	71.60	g
KH ₂ PO ₄	26.00	g
Distilled water	1000.00	ml

Dissolve in about 800 ml of water, adjust the pH to 6.8 and make up to 1 litre.

Prepare the growth medium as follows:

Dilute 100 ml solution 1 to 1 litre with distilled water and then add 1 ml of solution 3, 1 ml of the trace elements, and 0.1 ml of solution 2. If solid media is required add 1.5% agar. Dispense the medium into the growth vessels. If using sealed vessels it is appropriate to add 50% methane to the gas phase and autoclave at 115°C (15psi) for 15 minutes. Autoclave the phosphate buffer separately. When the growth medium is cool 10 ml/l of the phosphate buffer is added - if too warm the phosphate will precipitate. Liquid cultures should be grown with shaking.