

1376. METHYLOCOCCUS MEDIUM

KNO ₃	0.10	g
MgSO ₄ x 7 H ₂ O	0.10	g
CaCl ₂ x 2 H ₂ O	0.02	g
KBr	0.01	g
Trace element solution (see below)	0.10	ml
Vitamin solution (see below)	0.10	ml
Selenite-Tungstate Solution (see below)	0.10	ml
FeNa-EDTA (see below)	0.10	ml
Phosphate Buffer (see below)	2.00	ml
Distilled Water	1000.00	ml

Trace Element Solution:

<i>Na₂ EDTA</i>	<i>0.25</i>	<i>g</i>
<i>ZnSO₄ x 7 H₂O</i>	<i>0.40</i>	<i>g</i>
<i>MnCl₂ x 4 H₂O</i>	<i>0.02</i>	<i>g</i>
<i>H₃BO₃</i>	<i>0.02</i>	<i>g</i>
<i>Na₂MoO₄ x 2 H₂O</i>	<i>0.04</i>	<i>g</i>
<i>NiCl₂ x 2 H₂O</i>	<i>0.01</i>	<i>g</i>
<i>CuSO₄ x 5 H₂O</i>	<i>0.20</i>	<i>g</i>
<i>CoCl₂ x 6 H₂O</i>	<i>0.05</i>	<i>g</i>
<i>Distilled water</i>	<i>1000.00</i>	<i>ml</i>

Vitamin solution:

Thiamine-HCl x 2 H ₂ O	10.0	mg
Nicotinic acid	20.0	mg
Pyridoxamine-HCl	10.0	mg
p-aminobenzoic acid	10.0	mg
Riboflavin	20.0	mg
Biotin	1.0	mg
Vitamin B ₁₂	5.0	mg
Distilled Water	1000.0	ml

Selenite-Tungstate Solution:

NaOH	0.400	g
Na ₂ SeO ₃ x 5 H ₂ O	0.006	g
Na ₂ WO ₄ x 2 H ₂ O	0.008	g
Distilled Water	1000.000	ml

Phosphate Buffer:

KH ₂ PO ₄	37.425	g
Na ₂ HPO ₄	48.950	g
Distilled Water	1000.000	ml
pH 6.7		

FeNa-EDTA:

FeNa-EDTA	4.50	g
Distilled Water	1000.00	ml

Prepare the medium without the vitamin solution and the phosphate buffer. 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 121°C for 15 minutes. Autoclave the phosphate buffer separately. When the growth medium is cool add 0.1 ml/l of vitamin solution (filter sterilized) and 10 ml/l of the phosphate buffer is added - if too warm the phosphate will precipitate. Liquid cultures should be grown with shaking.