

2512

Revised Anaerobic Mineral Medium (RAMM)

KH₂PO₄, 0.27 g
K₂HPO₄, 0.35 g
NH₄Cl, 0.53 g
CaCl₂ * 2H₂O, 0.075 g
MgCl₂ * 6H₂O, 0.10 g
FeCl₂ * 4H₂O, 0.020 g
Trace minerals (see Below), 5.0 ml
DI water, 995.0 ml

The medium is boiled and cooled under a continuous stream of N₂. After cooling, the following ingredients are added to the final concentration from stock solutions.

Na₂S * 9 H₂O, 0.5 mg/L
Resazurin, 0.5 mg/L
NaHCO₃, 1.2 g/L

The pH of the medium is adjusted to 7.0-7.2. The medium should be dispense under a gas phase of 80% N₂ 20% CO₂. Autoclave for 20 minutes at 121 °C. After autoclaving, the following filter sterilized solutions are added.

Wolin Vitamin supplement (see Below), 1.0 ml
Vitamin B₁₂ (final concentration), 0.5 mg/L

For growth: add 40mM pyruvate and 40mM fumarate from stock solutions just before inoculation.

Trace minerals:

EDTA, 0.500 g
MgSO₄ · 7H₂O, 3.000 g
MnSO₄ · H₂O, 0.500 g
NaCl, 1.00 g
FeSO₄ · 7H₂O, 0.100 g
Co(NO₃)₂ · 6H₂O, 0.100 g
CaCl₂ (anhydrous), 0.100 g
ZnSO₄ · 7H₂O, 0.100 g
CuSO₄ · 5H₂O, 0.010 g

AlK(SO₄)₂ (anhydrous), 0.010 g
H₃BO₃, 0.010 g
Na₂MoO₄ · 2H₂O, 0.010 g
Na₂SeO₃ (anhydrous), 0.001 g
Na₂WO₄ · 2H₂O, 0.010 g
NiCl₂ · 6H₂O, 0.020 g
DI water 1000.0 ml

Wolin Vitamin Supplement:

p-aminobenzoic acid, 50.0 mg
Biotin, 50.0 mg
Cyanocobalamin (B₁₂), 1.0 mg
Folic acid, 20.0 mg
1,4-naphthoquinone, 50.0 mg
Nicotinamide, 50.0 mg
Pantothenic acid, 50.0 mg
Pyridoxine hydrochloride, 100.0 mg
Riboflavin, 50.0 mg
Thiamine, 50.0 mg
Thioctic acid, 50.0 mg