

1266. MEDIUM VL55

Liquid media:

MES (2-Morpholinoethanesulfonic acid)	1.95	g
20 mM MgSO ₄ x 7 H ₂ O	10.00	ml
30 mM CaCl ₂ x 2 H ₂ O	10.00	ml
20 mM (NH ₄) ₂ HPO ₄	10.00	ml
Selenite-tungstate solution (see below)	1.00	ml
Trace element solution SL10 (see below)	1.00	ml
Distilled water	960.00	ml

Adjust to pH 5.5 with NaOH/KOH solution (see below).

After autoclaving add:

Vitamin solution (see below)	3.00	ml
0.2M Glucose (filter-sterilized)	10.0	ml

Solid media:

MES (2-Morpholinoethanesulfonic acid)	3.90	g
20 mM MgSO ₄ x 7 H ₂ O	20.00	ml
30 mM CaCl ₂ x 2 H ₂ O	20.00	ml
20 mM (NH ₄) ₂ HPO ₄	20.00	ml
Selenite-tungstate solution (see below)	2.00	ml
Trace element solution SL10	2.00	ml
Distilled water	920.00	ml

Adjust to pH 5.5 with NaOH/KOH solution (see below).

Autoclave in 250ml lots, with a magnetic stirring bar in each bottle.

After autoclaving, keep at about 60°C, then add to each 250 ml lot:

Vitamin solution (see below)	1.50	ml
0.2M Glucose (filter-sterilized)	10.00	ml
3% washed Agar (autoclaved, kept about 60°C)	250.00	ml

Stocks for the media:

20 mM MgSO₄ x 7 H₂O:

H ₂ O	100.00	ml
MgSO ₄ x 7 H ₂ O	0.49	g

30 mM CaCl₂ x 2 H₂O:

H ₂ O	100.00	ml
CaCl ₂ x 2 H ₂ O	0.44	g

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20mM (NH₄)₂HPO₄:

H ₂ O	100.00	ml
(NH ₄) ₂ HPO ₄	0.26	g

NaOH/KOH:

H ₂ O	100.00	ml
NaOH	0.80	g
KOH	0.56	g

Selenite-tungstate solution:

NaOH	0.500	g
Na ₂ SeO ₃ x 5 H ₂ O	0.003	g
Na ₂ WO ₄ x 2 H ₂ O	0.004	g
Distilled water	1000.000	ml

Trace element solution SL-10:

HCl (25%; 7.7 M)	10.00	ml
FeCl ₂ x 4 H ₂ O	1.50	g
ZnCl ₂	70.00	mg
MnCl ₂ x 4 H ₂ O	100.00	mg
H ₃ BO ₃	6.00	mg
CoCl ₂ x 6 H ₂ O	190.00	mg
CuCl ₂ x 2 H ₂ O	2.00	mg
NiCl ₂ x 6 H ₂ O	24.00	mg
Na ₂ MoO ₄ x 2 H ₂ O	36.00	mg
Distilled water	990.00	ml

First dissolve FeCl₂ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.0 ml.

Vitamin solution:

Vitamin B ₁₂	17.0	mg
4-aminobenzoate	13.0	mg
Biotin	3.0	mg
Nicotinic acid	33.0	mg
Hemicalcium D-(+)- pantothenate	17.0	mg
Pyridoxamine-HCl	50.0	mg
Thiamine-HCl x 2 H ₂ O	33.0	mg
D,L-6,8-thioctic acid	10.0	mg
Riboflavin	10.0	mg
Folic acid	4.0	mg
Distilled water	1000.0	ml

Filter sterilize the solution.