

## 1266. MEDIUM VL55

### Liquid media:

MES (2-Morpholinoethanesulfonic acid)	1.95	g
20 mM MgSO <sub>4</sub> x 7 H <sub>2</sub> O	10.00	ml
30 mM CaCl <sub>2</sub> x 2 H <sub>2</sub> O	10.00	ml
20 mM (NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>	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 <sub>4</sub> x 7 H <sub>2</sub> O	20.00	ml
30 mM CaCl <sub>2</sub> x 2 H <sub>2</sub> O	20.00	ml
20 mM (NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>	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 MgSO<sub>4</sub> x 7 H<sub>2</sub>O:*

H <sub>2</sub> O	100.00	ml
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	0.49	g

#### *30 mM CaCl<sub>2</sub> x 2 H<sub>2</sub>O:*

H <sub>2</sub> O	100.00	ml
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.44	g

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*20mM (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub>:*

H <sub>2</sub> O	100.00	ml
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	0.26	g

*NaOH/KOH:*

H <sub>2</sub> O	100.00	ml
NaOH	0.80	g
KOH	0.56	g

*Selenite-tungstate solution:*

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

*Trace element solution SL-10:*

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

First dissolve FeCl<sub>2</sub> in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.0 ml.

*Vitamin solution:*

Vitamin B <sub>12</sub>	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 <sub>2</sub> 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.