

## ATCC Medium: 2734 VL55 Medium

### For Broth

MES.....	1.95 g
20 mM MgSO <sub>4</sub> .....	10 ml
30 mM CaCl <sub>2</sub> .....	10 ml
20 mM (NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub> .....	10 ml
Se/W.....	1 ml
SL-10.....	1 ml
DI Water.....	960 ml

pH w/ NaOH/KOH to 5.5 (about 6 ml)

After autoclaving, add:

Vitamin 10 mix.....	3 ml
0.2M glucose.....	10 ml

### For Agar

MES.....	3.9 g
20 mM MgSO <sub>4</sub> .....	20 ml
30 mM CaCl <sub>2</sub> .....	20 ml
20 mM (NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub> .....	20 ml
Se/W.....	2 ml
SL-10 (pH w/ NaOH/KOH).....	2 ml
DI Water.....	920 ml

pH w/ NaOH/KOH to 5.5 (about 12 ml)

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

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

Vitamin 10 mix.....	1.5 ml
0.2M glucose.....	10 ml
3% washed agar.....	250 ml (keep at about 60°C)

Stir and pour plate

NOTE: If the agar or gellan is added to the MES-containing medium, fewer bubbles will be formed.

### Stocks for VL55

20mM MgSO <sub>4</sub>	MgSO <sub>4</sub> x7H <sub>2</sub> O.....	0.49 g
	DI Water.....	100 ml

30mM CaCl <sub>2</sub>	CaCl <sub>2</sub> x2H <sub>2</sub> O.....0.44 g
	DI Water.....100 ml
20 mM (NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>	(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub> .....0.26 g
	DI Water.....100 ml
NaOH/KOH	NaOH.....0.80 g (200mM)
	KOH.....0.56 g (100mM)
	DI Water.....100 ml

### Washed Agar

Suspend about 10% more agar than finally required in a large volume of water (i.e., 30g in 2000ml of glass-distilled H<sub>2</sub>O), and stir with magnetic stirrer for about 5 min. Turn off stirrer, and allow agar to settle (about 30 min). Decant off supernatant and repeat this washing step about 5 times, to remove sugars etc from the agar. The supernatant will at first be yellowish, but later clearer. Resuspend agar in required final volume of water, and autoclave.

### Trace element solution SL10

The components of the trace element solutions SL10 (Widdel et al. 1983) are added and dissolved in the order listed.

25% HCl.....	10 ml
FeCl <sub>2</sub> x4H <sub>2</sub> O.....	1.5 g
CoCl <sub>2</sub> x6H <sub>2</sub> O.....	190 mg
MnCl <sub>2</sub> x4H <sub>2</sub> O.....	100 mg
ZnCl <sub>2</sub> .....	70 mg
H <sub>3</sub> BO <sub>3</sub> .....	6 mg
Na <sub>2</sub> MoO <sub>4</sub> x2H <sub>2</sub> O.....	36 mg
NiCl <sub>2</sub> x6H <sub>2</sub> O.....	24 mg
CuCl <sub>2</sub> x2H <sub>2</sub> O.....	2 mg
DI Water.....	1000 ml

The trace element solution is autoclaved under air, in 25 ml aliquots in 50 ml screw-capped bottles. Large stocks do not need to be sterilized for storage.

### Selenite/tungstate solution

NaOH.....	0.5 g
Na <sub>2</sub> SeO <sub>3</sub> x5H <sub>2</sub> O.....	3 mg
Na <sub>2</sub> WO <sub>4</sub> x2H <sub>2</sub> O.....	4 mg
DI Water.....	1000 ml

The selenite/tungstate solution (Tschech and Pfennig 1984) is autoclaved under air in 50 ml aliquots in 100 ml screw-capped bottles.

## Vitamin 10 stock

4-aminobenzoate.....	13 mg
d-(+)-biotin.....	3 mg
Nicotinic acid.....	33 mg
Hemicalcium D-(+)-pantothenate.....	17 mg
Pyridoxamine hydrochloride.....	50 mg
Thiamine hydrochloride.....	33 mg
Cyanocobalamin.....	17 mg
DL-6,8-thioctic acid.....	10 mg
Riboflavin.....	10 mg
Folic acid.....	4 mg

Vitamin 10 solution is sterilized by filtration into sterile bottles through sterile 0.2µm pore-size cellulose acetate filters. The bottles are wrapped in aluminum foil to protect against light, and stored at 4°C.