

## M22 (Dr. C Neil Hunter)

### 10X Stock:

			To make up 4 Litres
• Potassium dihydrogen orthophosphate	KH <sub>2</sub> PO <sub>4</sub>	122.4g	
• Diapotassium dihydrogen orthophosphate	K <sub>2</sub> HPO <sub>4</sub>	120.0g	
• DL – Lactic acid (fridge)	Na Lactate solution	100.0g	
• Ammonium sulphate – big pot	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	20g	
• Sodium Chloride	NaCl	20g	
• Sodium succinate		173.7g	
• Sodium glutamate	L – glutamic acid	10.8g	
• Aspartic acid	DL – aspartic acid	1.6g	
• Solution C		800ml	

Make up to 2-3 litres, pH to 6.8 and then make up to 4 Litres.  
Autoclave as 10 X 500ml flats.

### Solution C:

			Make up to 4 Litres
• Nitrilotriacetic acid (brown jar)			40g
• Magnesium Chloride	MgCl <sub>2</sub>	96g	
• Calcium Chloride	CaCl <sub>2</sub>	13.36g	
• EDTA		0.5g	
• Zinc Chloride (poison)	ZnCl <sub>2</sub>	1.044g	
• Ferrous Chloride (poison)	FeCl <sub>2</sub>	1.0g	
• Manganous Chloride	MnCl <sub>2</sub>	0.36g	
• Ammonium molybdate	(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> H <sub>2</sub> O	0.037g	
• Cupric Chloride (poison)	CuCl <sub>2</sub>	0.031g	
• Cobaltous nitrate (poison)	Co(NO <sub>3</sub> ) <sub>2</sub>	0.0496g	
• Boric acid (orthoboric acid)		0.0228g	

Do not autoclave, just freeze at -20°C in 400ml aliquots.

### Casamino acids (CAA):

		To make 1 Litre
• Casein Hydrosylate acid		50g

Makes up 5% solution to be aliquotted into 200ml.

### 1X M22:

		To make up 2 Litres
• 10X stock M22		200ml
• CAA		40ml
• Water		1760ml

- Batches - 1.5 Litres in 2 Litre flasks  
- 12 X 80ml in 100ml flasks  
- 100 X 10ml in universals.

For M22 agar add 1.5g agar to 100ml of M22 with no CAA in, store in 300ml flats.

### **Vitamin solution**

prepare a 10,000 times stock solution of vitamins as follows.

nicotinic acid	1g	
Thiamine	0.5g	
pABA(p-Aminobenzoic acid)	0.1g	
Biotin (d-Biotin)	0.01g	
Milli-Q water	100ml	

Aliquot into 20mls after filter sterilisation.

Add 1ul per every 10ml of M22 media. Do this after you have autoclaved the media, since the vitamins are labile and the heat will destroy them. When adding them to melted agar wait until the agar is relatively cool. Freeze the aliquots you aren't using and keep your working stock in the fridge.