

## Moss Methods Sheet

### MOSS MEDIA RECIPES - David Cove

(last update 10<sup>th</sup> August 2004)

#### BCD medium (routine basal medium)

stock solution B	10 ml
stock solution C	10 ml
stock solution D	10 ml
trace element solution	1 ml
agar	8 g
distilled H <sub>2</sub> O	to 1 l

Sterilise by autoclaving.

If required, di-ammonium (+) tartrate may be added before autoclaving (5 mM = 920 mg/l)

or if you prefer, you can do it this way:

Add directly to distilled water as follows:

	1 litre	5 litres	10 litres
Distilled water	1 litre	5 litres	10 litres
MgSO <sub>4</sub> .7H <sub>2</sub> O (or anhydrous MgSO <sub>4</sub> )	250 mg (120 mg)	1.25 g (600 mg)	2.5 g (1.2 g)
KH <sub>2</sub> PO <sub>4</sub>	250 mg	1.25 g	2.5 g
KNO <sub>3</sub>	1.01 g	5.05 g	10.1 g
FeSO <sub>4</sub> .7H <sub>2</sub> O	12.5 mg	62.5 mg	125 mg
trace element solution	1 ml	5 ml	10 ml
agar	8 g	40 g	80 g

adjust pH to 6.5 with 4 M KOH (before addition of agar). Sterilise by autoclaving.

Either way you will need to add CaCl<sub>2</sub> to 1 mM immediately before pouring plates.

CaCl<sub>2</sub> may be kept as a 1M stock solution (sterilised by autoclaving).

#### PRMB (protoplast regeneration medium – bottom layer)

stock solution B	10 ml
stock solution C	10 ml
stock solution D	10 ml
trace element solution	1 ml
D-mannitol	60 g
di-ammonium (+) tartrate	920 mg (=5mM)
agar	10 g
distilled H <sub>2</sub> O	to 1 l

Sterilise by autoclaving.

Add CaCl<sub>2</sub> to a final concentration of 10 mM before use.

PRMT (protoplast regeneration medium – top layer)

stock solution B	10 ml	
stock solution C	10 ml	
stock solution D	10 ml	
trace element solution	1 ml	
D-mannitol	80 g	
di-ammonium (+) tartrate	920 mg	(=5mM)
agar	6 g	
distilled H <sub>2</sub> O	to 1 l	

Sterilise by autoclaving.

Add CaCl<sub>2</sub> to a final concentration of 10 mM before use.

PRML (liquid protoplast regeneration medium)

stock solution B	10 ml	
stock solution C	10 ml	
stock solution D	10 ml	
trace element solution	1 ml	
D-mannitol	60 g	
di-ammonium (+) tartrate	920 mg	(=5mM)
distilled H <sub>2</sub> O	to 1 l	

Sterilise by autoclaving.

Add CaCl<sub>2</sub> to a final concentration of 10 mM before use.

Stock solutions for media making

Solution B

MgSO <sub>4</sub> .7H <sub>2</sub> O	25 g	(anhydrous MgSO <sub>4</sub> 12 g)
distilled H <sub>2</sub> O	to 1 l	

Solution C

KH <sub>2</sub> PO <sub>4</sub>	25 g	
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distilled H<sub>2</sub>O 500 ml

Adjust pH to 6.5 with minimal volume of 4 M KOH

Make up to 1 l with additional distilled H<sub>2</sub>O

#### Solution D

KNO<sub>3</sub> 101 g  
 FeSO<sub>4</sub>·7H<sub>2</sub>O 1.25 g  
 distilled H<sub>2</sub>O to 1 l

#### Trace element solution

H <sub>3</sub> BO <sub>3</sub>	614 mg	MnCl <sub>2</sub> ·4H <sub>2</sub> O	389 mg
AlK(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O	110 mg	CoCl <sub>2</sub> ·6H <sub>2</sub> O	55 mg
CuSO <sub>4</sub> ·5H <sub>2</sub> O	55 mg	ZnSO <sub>4</sub> ·7H <sub>2</sub> O	55 mg
KBr	28 mg	KI	28 mg
LiCl	28 mg	SnCl <sub>2</sub> ·2H <sub>2</sub> O	28 mg
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	25 mg	NiCl <sub>2</sub> ·6H <sub>2</sub> O	59 mg
distilled H <sub>2</sub> O.	to 1 l		

We use this trace element solution but others have used other recipes, the exact composition of the trace element solution is probably not important.

#### Growth Supplements

<u>substance</u>	<u>concentration</u> <u>in the medium</u>	<u>weight</u> <u>per litre</u>	<u>stock</u> <u>sol'n</u>
p-aminobenzoic acid	1.8 µM	247 µg	_ 1000
di-ammonium (+) tartrate	5 mM	920 mg	_ 100
CaCl <sub>2</sub> ·2H <sub>2</sub> O	1 or 10 mM	147 mg or 1.47 g	_ 1000
CaCl <sub>2</sub> ·6H <sub>2</sub> O	1 or 10 mM	219 mg or 2.19 g	_ 1000
D-glucose	0.5% (w/v) (= 28 mM)	5 g	_ 100
nicotinic acid	8 µM	1 mg	_ 1000
D-sucrose	0.5% (w/v) (= 15 mM)	5 g	_ 100
thiamine HCl	1.5 µM	0.5 mg	_ 1000

#### References

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