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## BG-11 media

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**We use this protocol and it's working**

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## Abstract

From McCormick lab, University of Edinburgh

(written by Anja Nenninger, Grant Gale, Alejandra Schiavon and Anton Puzorjov), introduced to protocols.io by myself



## Stock solutions

### 1 **BG11 media**

100XBG11:

Ingredient	Quantity (g/L)	
NaNO <sub>3</sub>	149.6	
MgSO <sub>4</sub> 7H <sub>2</sub> O	7.49	
CaCl <sub>2</sub>	3.6	
Citric acid	0.6	
Na <sub>2</sub> EDTA	1.12 ml 0.25M solution, pH 8.0	0.61 mL 0.5 M

### Trace elements

A	B	C
Ingredient	Quantity (g/L)	
H <sub>3</sub> BO <sub>3</sub>	2.86	
MnCl <sub>2</sub> 4H <sub>2</sub> O	1.81	
ZnSO <sub>4</sub> 7H <sub>2</sub> O	0.22	
Na <sub>2</sub> MoO <sub>4</sub> 2H <sub>2</sub> O	0.39	
Co(NO <sub>3</sub> ) <sub>2</sub> 6H <sub>2</sub> O	0.05	
CuSO <sub>4</sub> 5H <sub>2</sub> O	0.08	

### Iron stock

Ingredient	Quantity (g/100 mL)
Ferric ammonium citrate	1.11



### Phosphate stock

Ingredient	Quantity (g/100 mL)
K <sub>2</sub> HPO <sub>4</sub>	3.05

### Glucose stock

Ingredient	Quantity (g/100 mL)
Glucose	9

### Sodium carbonate stock

Ingredient	Quantity (g/100 mL)
Sodium carbonate	2

Autoclave all above solutions

Filter sterilize all below solutions

HEPES buffer

Ingredient	Quantity (g/100 mL)
HEPES	23.8

Adjust to pH 7.8 using NaOH



## Sodium bicarb stock

Ingredient	Quantity (g/100 mL)
NaHCO <sub>3</sub>	8.4

## Liquid BG11

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### 2. Liquid BG11 growth medium

For 1 litre BG11:

10 ml 100x BG11

1 ml Trace elements

1 ml Iron stock

1 ml Na<sub>2</sub>CO<sub>3</sub>

1 ml Phosphate stock

10 ml of TES (pH 8.2)

Up to 990ml with dH<sub>2</sub>O

Autoclave

10 ml NaHCO<sub>3</sub> stock (added after cooling).

Note: If the NaHCO<sub>3</sub> is added before autoclaving it can cause undesired precipitation.

Note: For large scale projects 10 ml of TES can be replaced with 5 ml of HEPES to reduce the cost

Note: Mix properly before use, iron tends to precipitate.

Optional extras:



- 10 ml Glucose stock (e.g. essential for mutants without functional PSII. Final glucose concentration = 5 mM).
  - Antibiotics (see point 6).
- Note: If you add optional extras remember to adjust the volume of the dH<sub>2</sub>O accordingly.

## BG11 agar

- 3 3. BG11 agar plates  
For 1 litre BG11 agar:  
To prepare BG11 agar plates the growth medium and the agar must be autoclaved separately.  
\*Prepare in 1L bottle  
10 ml 100x BG11  
1 ml Trace elements  
1 ml Iron stock  
1 ml Na<sub>2</sub>CO<sub>3</sub>  
1 ml Phosphate stock  
10 ml of TES (pH 8.2)  
**3 g of sodium thiosulphate**  
Top up to 490ml with dH<sub>2</sub>O,

Autoclave

\*Prepare in 500 ml bottle  
15 g of Difco bacto-agar  
Top up to 500ml with dH<sub>2</sub>O

Autoclave

Wait until both are ~60°C, mix and add:  
10 ml NaHCO<sub>3</sub> stock  
Antibiotic if necessary

## BG11 LB agar

- 4 For 1 litre BG11 + LB agar:



To prepare BG11 + LB agar plates the growth medium and the must will be autoclaved separately.

\*Prepare in 1L bottle

10 ml 100x BG11

1 ml Trace elements

1 ml Iron stock

1 ml Na<sub>2</sub>CO<sub>3</sub>

1 ml Phosphate stock

Top up to 440ml with dH<sub>2</sub>O,

Autoclave

\*Prepare in 500 ml bottle

15 g of Difco bacto-agar

Top up to 500ml with dH<sub>2</sub>O

Autoclave

Wait until both are ~60°C, mix and add:

10 ml NaHCO<sub>3</sub> stock

50 ml of sterile LB medium (premade from the media kitchen)

Note: These plates do not require the addition of 3g of thiosulphate and TES buffer since they are commonly used only for 24 hr after triparental-mating (conjugation)

## BG11 Sucrose

5 For 1 litre BG11 + Sucrose agar:

To prepare BG11 + sucrose agar plates the growth medium, the agar and the sucrose will be autoclaved separately.

\*Prepare in 1L bottle

10 ml 100x BG11

1 ml Trace elements

1 ml Iron stock

1 ml Na<sub>2</sub>CO<sub>3</sub>

1 ml Phosphate stock

10 ml of TES (pH 8.2)

3 g of sodium thiosulphate

Top up to 400ml with dH<sub>2</sub>O,

Autoclave



\*Prepare in 500 ml bottle  
15 g of Difco bacto-agar  
Top up to 500ml with dH<sub>2</sub>O

Autoclave

\*Prepare in 200 ml bottle  
50 g of Sucrose  
Top up to 100ml with dH<sub>2</sub>O  
Autoclave

Wait until all are ~60°C and mix.

Note: Once you have poured the plates wrap them individually with parafilm since they are very prone to contamination.

## Antibiotics

### 6

#### 6. Antibiotics

Add after medium is autoclaved

Chloramphenicol 25 µg/ml. Use 25 mg/ml in ethanol stock, add 1ml/L.

Spectinomycin 50 µg/ml. Use 100 mg/ml in water stock, add 0.5ml/L.

Kanamycin 50 µg/ml. Use 50 mg/ml in water stock, add 1 ml/L.

Erythromycin 50 µg/ml. Use 50 mg/ml in ethanol stock, add 1 ml/L.

Note: Antibiotic quantities above are for 1 litre of media. If your medium volume is different, add the appropriate concentration of antibiotics.