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BG-11 media

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Protocol status: Working
We use this protocol and it's working

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Disclaimer

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)	
	NaNO3	149.6	
	MgSO4 7H2O	7.49	
	CaCl2	3.6	
	Citric acid	0.6	
	Na2EDTA	1.12 ml 0.25M solution, pH 8.0	0.61 mL 0.5 M

Trace elements

	A	B	C
	Ingredient	Quantity (g/L)	
	H3BO3	2.86	
	MnCL2 4H2O	1.81	
	ZnSO4 7H2O	0.22	
	Na2MoO4 2H2O	0.39	
	Co(NO3)2 6H2O	0.05	
	CuSO4 5H2O	0.08	

Iron stock

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

Phosphate stock

Ingredient	Quantity (g/100 mL)
K2HPO4	3.05

Glucose stock

Ingredient	Quantity (g/100 mL)
Glucose	9

Soidum carbonate stock

Ingredient	Quantity (g/100 mL)
Soidum 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)
NaHCO3	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 Na2CO3
1 ml Phosphate stock
10 ml of TES (pH 8.2)
Up to 990ml with dH2O

Autoclave

10 ml NaHCO3 stock (added after cooling).

Note: If the NaHCO3 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 dH2O 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 Na2CO3
1 ml Phosphate stock
10 ml of TES (pH 8.2)
3 g of sodium thiosulphate
Top up to 490ml with dH2O,

Autoclave

*Prepare in 500 ml bottle
15 g of Difco bacto-agar
Top up to 500ml with dH2O

Autoclave

Wait until both are ~60°C, mix and add:
10 ml NaHCO3 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 Na2CO3
1 ml Phosphate stock
Top up to 440ml with dH2O,

Autoclave

*Prepare in 500 ml bottle
15 g of Difco bacto-agar
Top up to 500ml with dH2O

Autoclave

Wait until both are ~60°C, mix and add:
10 ml NaHCO3 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 Na2CO3
1 ml Phosphate stock
10 ml of TES (pH 8.2)
3 g of sodium thiosulphate
Top up to 400ml with dH2O,

Autoclave

*Prepare in 500 ml bottle
15 g of Difco bacto-agar
Top up to 500ml with dH2O

Autoclave

*Prepare in 200 ml bottle
50 g of Sucrose
Top up to 100ml with dH2O
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

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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.