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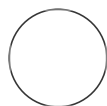
🌐 BG11_and_inducer_preparation

📁 In 1 collection

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ABSTRACT

Protocol for the preparation of media and inducers used during the interlaboratory study published by Mager et al. 2023.

BG11-PC overview

1 BG11 medium for interlab study (after van Alphen et al., 2018)

Macronutrients	mM	g/L
NaNO ₃	17.65	1.5
KH ₂ PO ₄	0.294	0.04
MgSO ₄ * 7H ₂ O	0.304	0.075
CaCl ₂ * 2H ₂ O	0.244	0.036
FeCl ₃ * 6H ₂ O	0.015	0.004
Na ₂ EDTA * 2 H ₂ O	0.05	0.0186
NaHCO ₃	9.44	1
HEPES	5	1.2
Micronutrients	μM	mg/L
H ₃ B ₃ O ₃	46.25	2.86
MnCl ₂ * 4H ₂ O	9.15	1.81
ZnSO ₄ * 7H ₂ O	0.77	0.222
Na ₂ MoO ₄ * 2H ₂ O	1.62	0.391
CuSO ₄ * 5H ₂ O	0.316	0.079
Co(NO ₃) ₂ 6H ₂ O	0.168	0.049

Molarity and mass concentration of all BG11 media components after van Alphen et al. 2018
CuSO₄ was omitted for the Interlab study to have a copper free media.

Note

van Alphen P, Abedini Najafabadi H, Branco dos Santos F, Hellingwerf KJ. Increasing the Photoautotrophic Growth Rate of *Synechocystis* sp. PCC 6803 by Identifying the Limitations of Its Cultivation. *Biotechnol J*. 2018 Aug;13(8):1700764.

DOI: [10.1002/biot.201700764](https://doi.org/10.1002/biot.201700764)

1.1

Component	mL/L
Buffer solution	5 mL
Stock 1	2.5 mL
Stock 2	2.5 mL
Stock 3	2.5 mL

Stock 4	10.5 mL
Fill with autoclaved water to 1L	

Overview over the stock solutions used to create the final BG11 media

1.2

BG-11 is split into four stock solutions such that incompatible (i.e. because of low solubility) compounds at high concentration are avoided. Additionally, a minimal amount of HEPES buffer is added to ensure the same starting pH of the cultures.

The final medium is prepared by adding buffer and each stock solution to 500 ml autoclaved water measuring cylinder and filling it to a total of 1 liter.

HEPES buffer preparation

2

A	B
HEPES buffer solution	g/L
HEPES, NaOH (pH 8)	240

Mass concentration of HEPES buffer solution

2.1 Dissolve 24g of HEPES in 40 ml of MilliQ water

2.2 Under stirring adjust pH to 8 using NaOH

2.3 Add MilliQ water to 100 ml

2.4 Filter sterilize the solution

Stock solution 1 preparation

3

Stock 1	g/L
NaNO ₃	600
CaCl ₂ * 2H ₂ O	14.4

Mass concentration of stock solution 1

3.1 Dissolve 1.4g CaCl₂*2H₂O in 70 ml of MilliQ water

3.2 Add 60g of NaNO₃ under vigorous stirring at 50°C until fully dissolved.

3.3 Cool solution to room temperature and add MilliQ water to 100 ml.

3.4 Filter sterilize the solution

Stock solution 2 preparation

4

A	B
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A	B
Stock 2	g/L
MgSO ₄ * 7H ₂ O	30
FeCl ₃ * 6H ₂ O	1.62
Na ₂ EDTA * 2 H ₂ O	2.24
Micronutrients	40 mL in 100 mL

Mass concentration of stock solution 2

- 4.1 Dissolve 0.224g EDTA Na₂*2H₂O in 40 mL MilliQ water followed by 0.162g FeCl₃·6H₂O and leave the solution stirring over night
- 4.2 Dissolve 3g MgSO₄*7H₂O in 15 ml MilliQ water and add it to the EDTA Na₂*2H₂O + FeCl₃·6H₂O solution
- 4.3 Add 40 ml of Micronutrient solution (See Section Micronutrient stock solution preparation) to this mix and fill to 100 ml using MilliQ water
- 4.4 Filter sterilize the solution.

Stock solution 3 preparation

5

Stock 3	g/L
KH ₂ PO ₄	16
Na ₂ EDTA * 2 H ₂ O	5.2

Mass concentration of stock solution 3

- 5.1 Dissolve 1.6 g K_2HPO_4 in 40 ml of MilliQ water
- 5.2 Dissolve 0.52 g $\text{EDTA Na}_2 \cdot 2\text{H}_2\text{O}$ in the same solution
- 5.3 Fill to 100 ml using MilliQ water
- 5.4 Filter sterilize the solution

Stock solution 4 preparation

6

Stock 4	g/L
NaHCO_3	80

Mass concentration of stock solution 4

- 6.1 Dissolve 8 g NaHCO_3 in 80 ml MilliQ water
- 6.2 Fill to 100 ml using MilliQ water

6.3 Filter sterilize the solution

Micronutrient stock solution preparation

7

A	B
Micronutrients	g/L
H ₃ BO ₃	2.86
MnCl ₂ * 4H ₂ O	1.81
ZnSO ₄ * 7H ₂ O	0.222
Na ₂ MoO ₄ * 2H ₂ O	0.391
CuSO ₄ * 5H ₂ O	0.079*
Co(NO ₃) ₂ 6H ₂ O	0.049

Mass concentration of Micronutrient stock solution

* Copper sulfate is not to be added for the interlab study as we need copper free media for PpetE induction

7.1 Add trace metal salts in the order they are listed to 100 ml of MilliQ water and wait for complete dissolution between each addition

7.2 Filter sterilize the solution

Rhamnose stock preparation

8

A	B	C
Rhamnose stock solution	g/L	mM
Rhamnose	164	1000

Mass concentration of Rhamnose inducer stock solution 1

8.1 Dissolve 8.2 g of rhamnose in 40 ml MilliQ water

8.2 Add MilliQ water to 50ml

8.3 Filter sterilize the solution

Copper sulfate stock preparation

9	A	B	C
	CuSO4 stock solution	g/L	mM
	CuSO4*5H2O	0.0025	0.1

Mass concentration of copper sulfate inducer stock solution

9.1 Dissolve 25 mg CuSO4*5H2O in 8 ml MiliQ water

9.2 Add to 10 ml using MilliQ water

9.3 Dilute 10 μ l of this solution in 9.99 ml MilliQ water

9.4 Filter sterilize the solution