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Recipe for standard BG-11 media

Forked from Recipe for standard BG-11 media

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We use this protocol and it's working

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Abstract

Stanier RY, Deruelles J, Rippka R, Herdman M, Waterbury JB: **Generic Assignments, Strain Histories and Properties of Pure Cultures of Cyanobacteria.** Microbiology 1979, 111:1–61.
Recipes for standard and alternative BG11 for culturing freshwater cyanobacteria, such as *Synechocystis* sp. PCC 6803, as described.
Media is usually not suitable for marine cyanobacteria.
Final Concentration of Medium.

CaCl2*2 H2O	0.036 g/L
Citric acid	0.006 g/L
NaNO3	1.4958 g/L
MgSO4* 7 H2O	0.0749 g/L
0.25M Na2EDTA (pH 8)	0.0056 mL/L
Na2CO3	20 µg/ml
Fe(III) Ammonium citrate	6 µg/ml
K2HPO4 * 3H2O	30 µg/ml
TES Buffer (pH 8)	10 mM
H3BO3	2.86 mg/L
MnCl2 * 4 H2O	1.81 mg/L
ZnSO4 * 7 H2O	0.222 mg/L
Na2MoO4 * 2 H2O	0.390 mg/L
Co(NO3)2 *6 H2O	0.049 mg/L
(CuSO4 * 5 H2O	0.079 mg/L if required)

Guidelines

Always work under sterile conditions when handling sterile media or stocks. Work under the clean bench.

Safety warnings

Wear gloves when preparing stocks!
Heavy metals are toxic for the environment and need to be discarded accordingly.

Before start

For plates:
Thaw antibiotic stocks before pouring plates.

100 x BG11 stock:

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- $CaCl_2$ $2H_2O$ (3.6 g · L^{-1})
- Citric acid (0.6 g · L^{-1})
- $NaNO_3$ (149.58 g · L^{-1})
- $MgSO_4$ · 7 H_2O (7.49 g · L^{-1})
- 0.25 M Na_2-EDTA, pH 8.0 (0.56 ml · L^{-1})

1.1 For BG11-N prepare a 50 X stock solution without NaNO3:

- CaCl2 × 2 H2O: 1.8 g/L
- Citric acid: 0.3 g/L
- MgSO4 × 7 H2O: 3.75 g/L
- 0.25 M Na2-EDTA, pH8.0: 0.28 ml/L

Supplemental stocks for standard media:

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- 1000x Na_2CO_3: 20 mg mL^{-1}
- 100x TES-buffer, pH 8.0 (1M), adjust with KOH
- 1000x K_2HPO_4 x 3 H_2O: 30 mg · mL^{-1}
- 1000x Fe(III) ammonium citrate (6 mg ·m L^{-1})
- 5000x $CuSO_4$ 5 H_2O (395 ng · mL^{-1}) (sterilize using a filter)

Trace metal mix:

3 1000x concentration:

- H_3BO_3 (2.86 g · L^{-1})
- $MnCl_2$ · 4 H_2O (1.81 g · L^{-1})
- $ZnSO_4$ · 7 H_2O (0.222 g · L^{-1})
- Na_2MoO_4 · 2 H_2O (0.390 g · L^{-1})
- $Co(NO_3)_2$ · 6 H_2O (0.049 g · L^{-1})

For BG11 lacking certain metals (e.g. for working with metal inducible promoters P_{petE}, P_{coaT}, P_{ziaA} etc., trace metal mix can be prepared lacking these chemicals and used instead of standard trace metal mix.

Standard 1x BG11

4 Fill 1 L bottle with 500 mL ultra pure water. Add stock solutions as shown below.

	Stock solution	Volume
	100x BG11 Stock	10 mL
	1000x Na_2CO_3	1 mL
	1000x K_2HPO_4 x 3 H_2O	1 mL
	100x TES-buffer	10 mL
	1000x Trace Metal Mix	1 mL

Add ultra pure water to 1 L.
Autoclave.
After autoclaving, add 1 mL 1000x Fe(III) ammonium citrate.
Optional: After autoclaving, add 200 µL 5000x $CuSO_4$

Standard 1x BG11 -N

5 Fill 1 L bottle with 500 mL ultra pure water. Add stock solutions as shown below.

	A	B
	Stock solution	**Volume**
	50x BG11 Stock -N	20 mL
	1000x Na_2CO_3	1 mL
	1000x K_2HPO_4 x 3 H_2O	1 mL
	100x TES-buffer	10 mL
	1000x Trace Metal Mix	1 mL

Add ultra pure water to 1 L.
Autoclave.
After autoclaving, add 1 mL sterile 1000x Fe(III) ammonium citrate.
Optional: After autoclaving, add 200 µL sterile 5000x $CuSO_4$

Standard 2x BG11 for agar plates

6 Fill 500 mL bottle with 250 mL ultra pure water. Add stock solutions as shown below.

	Stock solution	Volume
	100x BG11 Stock -N	10 mL
	1000x Na_2CO_3	1 mL
	1000x K_2HPO_4 x 3 H_2O	1 mL
	100x TES-buffer, pH = 8.0	10 mL
	1000x Trace Metal Mix	1 mL

Add ultra pure water to 500 mL.
Autoclave.
After autoclaving, add 1 mL sterile 1000x Fe(III) ammonium citrate.
Optional: After autoclaving, add 200 µL sterile 5000x $CuSO_4$

BG11 plates

7 Prepare 1.5 % agar: Weigh 4.5 g Bacto Agar. Fill up to 300 mL. Autoclave.

Microwave agar until liquid. Let cool.

8 In a 50 mL Falcon, add 1 vol 2x BG11 and 1 vol liquid 1.5 % agar. (Note: Usually, one plate requires 30-40 mL total volume.)

9 When mixture is hand warm, add appropriate antibiotics, if required. Quickly pour plate, avoiding air bubbles.