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# Recipe for standard BG-11 media

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1



[dx.doi.org/10.17504/protocols.io.bzjup4nw](https://dx.doi.org/10.17504/protocols.io.bzjup4nw)

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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.

CaCl <sub>2</sub> *2 H <sub>2</sub> O	0.036 g/L
Citric acid	0.006 g/L
NaNO <sub>3</sub>	1.4958 g/L
MgSO <sub>4</sub> * 7 H <sub>2</sub> O	0.0749 g/L
0.25M Na <sub>2</sub> EDTA (pH 8)	0.0056 mL/L
Na <sub>2</sub> CO <sub>3</sub>	20 µg/ml
Fe(III) Ammonium citrate	6 µg/ml
K <sub>2</sub> HPO <sub>4</sub> * 3H <sub>2</sub> O	30 µg/ml
TES Buffer (pH 8)	10 mM
H <sub>3</sub> BO <sub>3</sub>	2.86 mg/L
MnCl <sub>2</sub> * 4 H <sub>2</sub> O	1.81 mg/L
ZnSO <sub>4</sub> * 7 H <sub>2</sub> O	0.222 mg/L
Na <sub>2</sub> MoO <sub>4</sub> * 2 H <sub>2</sub> O	0.390 mg/L
Co(NO <sub>3</sub> ) <sub>2</sub> *6 H <sub>2</sub> O	0.049 mg/L
(CuSO <sub>4</sub> * 5 H <sub>2</sub> O	0.079 mg/L if required)

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## Recipe for standard BG-11 media, Thomas Rohr

cyanobacteria, Synechocystis, Synechococcus, culture, media, medium, BG11

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

Wear gloves when preparing stocks!

Heavy metals are toxic for the environment and need to be discarded accordingly.

For plates:

Thaw antibiotic stocks before pouring plates.

### 100 x BG11 stock:

- 1
  - $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  ( $3.6 \text{ g} \cdot \text{L}^{-1}$ )
  - Citric acid ( $0.6 \text{ g} \cdot \text{L}^{-1}$ )
  - $\text{NaNO}_3$  ( $149.58 \text{ g} \cdot \text{L}^{-1}$ )
  - $\text{MgSO}_4 \cdot 7 \text{ H}_2\text{O}$  ( $7.49 \text{ g} \cdot \text{L}^{-1}$ )
  - 0.25 M  $\text{Na}_2\text{-EDTA}$ , pH 8.0 ( $0.56 \text{ ml} \cdot \text{L}^{-1}$ )

### 1.1 For BG11-N prepare a 50 X stock solution without $\text{NaNO}_3$ :

- $\text{CaCl}_2 \cdot 2 \text{ H}_2\text{O}$ : 1.8 g/L
- Citric acid: 0.3 g/L
- $\text{MgSO}_4 \cdot 7 \text{ H}_2\text{O}$ : 3.75 g/L
- 0.25 M  $\text{Na}_2\text{-EDTA}$ , pH8.0: 0.28 ml/L

### Supplemental stocks for standard media:

- 2
  - 1000x  $\text{Na}_2\text{CO}_3$ :  $20 \text{ mg mL}^{-1}$
  - 100x TES-buffer, pH 8.0 (1M), adjust with KOH
  - 1000x  $\text{K}_2\text{HPO}_4 \cdot 3 \text{ H}_2\text{O}$ :  $30 \text{ mg} \cdot \text{mL}^{-1}$

- 1000x Fe(III) ammonium citrate ( $6 \text{ mg} \cdot \text{mL}^{-1}$ )
- 5000x  $\text{CuSO}_4 \cdot 5 \text{ H}_2\text{O}$  ( $395 \text{ ng} \cdot \text{mL}^{-1}$ ) (sterilize using a filter)

#### Trace metal mix:

- 3 1000x concentration:
- $\text{H}_3\text{BO}_3$  ( $2.86 \text{ g} \cdot \text{L}^{-1}$ )
  - $\text{MnCl}_2 \cdot 4 \text{ H}_2\text{O}$  ( $1.81 \text{ g} \cdot \text{L}^{-1}$ )
  - $\text{ZnSO}_4 \cdot 7 \text{ H}_2\text{O}$  ( $0.222 \text{ g} \cdot \text{L}^{-1}$ )
  - $\text{Na}_2\text{MoO}_4 \cdot 2 \text{ H}_2\text{O}$  ( $0.390 \text{ g} \cdot \text{L}^{-1}$ )
  - $\text{Co}(\text{NO}_3)_2 \cdot 6 \text{ H}_2\text{O}$  ( $0.049 \text{ g} \cdot \text{L}^{-1}$ )

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 $\text{Na}_2\text{CO}_3$	1 mL
1000x $\text{K}_2\text{HPO}_4 \cdot 3 \text{ H}_2\text{O}$	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  $\mu\text{L}$  5000x  $\text{CuSO}_4$

#### Standard 1x BG11 -N

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

A	B
<b>Stock solution</b>	<b>Volume</b>
50x BG11 Stock -N	20 mL
1000x Na <sub>2</sub> CO <sub>3</sub>	1 mL
1000x K <sub>2</sub> HPO <sub>4</sub> x 3 H <sub>2</sub> O	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<sub>4</sub>

#### Standard 2x BG11 for agar plates

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

<b>Stock solution</b>	<b>Volume</b>
100x BG11 Stock -N	10 mL
1000x Na <sub>2</sub> CO <sub>3</sub>	1 mL
1000x K <sub>2</sub> HPO <sub>4</sub> x 3 H <sub>2</sub> O	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<sub>4</sub>

#### 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.

