

Recipe for standard BG-11 media

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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.

Citation: Anna Behle Recipe for standard BG-11 media. **protocols.io**

dx.doi.org/10.17504/protocols.io.gtmbwk6

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Guidelines

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

Before start

For plates:

Thaw antibiotic stocks before pouring plates.

Protocol

100 x BG11 stock:

Step 1.

- $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ($3.6 \text{ g} \cdot \text{L}^{-1}$)
- Citric acid ($0.6 \text{ g} \cdot \text{L}^{-1}$)
- 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 ($0.56 \text{ ml} \cdot \text{L}^{-1}$)

For 100x BG11 Stock -N:

- Omit NaNO_3 .

Supplemental stocks for standard media:

Step 2.

- 1000x Na_2CO_3 : 20 mg L^{-1}
- 100x TES-buffer, pH 8.0 (1M), adjust with KOH
- 1000x $\text{K}_2\text{HPO}_4 \times 3 \text{ H}_2\text{O}$: $30 \text{ mg} \cdot \text{mL}^{-1}$
- 1000x Fe(III) ammonium citrate ($6 \text{ mg} \cdot \text{L}^{-1}$)
- 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:

Step 3.

1000x concentration:

- H_3BO_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

Step 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 $\text{K}_2\text{HPO}_4 \times 3 \text{ H}_2\text{O}$	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 µL 5000x CuSO₄

Standard 1x BG11 -N

Step 5.

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

Stock solution	Volume
100x BG11 Stock -N	10 mL
1000x Na ₂ CO ₃	1 mL
1000x K ₂ HPO ₄ x 3 H ₂ 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 CuSO₄

Standard 2x BG11 for agar plates

Step 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 ₂ CO ₃	1 mL
1000x K ₂ HPO ₄ x 3 H ₂ 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 CuSO₄

BG11 plates

Step 7.

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

Microwave agar until liquid. Let cool.

Step 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.)

Step 9.

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

Warnings

Wear gloves when preparing stocks!

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