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CD Media for High Density Cultivation of *Synechocystis* sp. PCC 6803

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1 *Works for me* dx.doi.org/10.17504/protocols.io.2bxgapn

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ABSTRACT

CellDeg's High Density Cultivation system allows for efficient accumulation of biomass of photosynthetic organisms, such as cyanobacteria and microalgae. The media requires a higher nutrient concentration, to facilitate high growth rates over extended periods. This protocol describes the media composition used in the Lindberg lab at Ångström laboratory (Uppsala University). The basic composition was previously described in Lippi et al., [1], and has been slightly modified:

- different iron source used
- $NaNO_3$ as sole (major) nitrogen source (no additional KNO_3)
- no cobalt is added to the standard media
- no copper is added to the standard media

[1] Lippi L, Bähr L, Wüstenberg A, Wilde A, Steuer R: Exploring the potential of high-density cultivation of cyanobacteria for the production of cyanophycin. Algal Research 2018, 31:363-366

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Dienst D., Wichmann J., Mantovani O., Rodrigues J., Lindberg P. (2019) High density cultivation for efficient sesquiterpenoid biosynthesis in Synechocystis sp. PCC 6803.

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1 **Prepare the following 8 stock solutions**

Stock	Components	Concentration	Stock concentration (g/L)
Stock A	K2HPO4	4 M	696.8
Stock B	MgSO4 + 7xH2O	2 M	493.0
Stock C	CaCl2 + 2xH20	500 mM	73.5
Stock D	Fe-ammonium-citrate (Ammonium iron(III) citrate)	150 mM	39.3
Stock E	NaNO3	5 M	425
Stock F	KHCO3	2.5 M	250.4
EDTA	Na2EDTA	150 mM	55.8
Trace elements	H3BO3	25 mM	1.55
	MnCl2 + 4xH2O	20 mM	4.0
	ZnSO4 + 2xH2O	2 mM	0.58
	Na2MoO4 + 2xH2O	3 mM	0.73
	CuSO4 + 5xH2O	20 µM	0.0005
	Co(NO3)2 + 5xH2O	60 µM	0.016

- **Filter sterilize** all solutions and store them at 4-10 °C (fridge)

If you are working with a promoter that is induced by metals (e.g. Zn^{2+}, Cu^{2+}, Co^{2+}), you can omit that salt from the Trace elements mix and supply it from a sterile filtered stock solution on demand.

Synechocystis 6803 can grow to high densities without any Cu^{2+} and Co^{2+} in the CD medium!

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2 Preparation of 1 L CD medium

- fill ~500 mL H_2O into an autoclavable 1 L bottle
  → do not mix the stock solution without excess H_2O to prevent salt precipitation
- add the following stock solutions before sterilization

Stock A	-	1 mL
Stock B	-	1 mL
Stock C	-	1 mL
Stock E	**-**	**10 mL**
EDTA	-	1 mL
Trace elements	-	1 mL

- add ddH_2O to a final volume of 1 L
- autoclave according to your standard procedure

- autoclaving leads to a significant loss of media volume!
  ⇒ Fill up the media with autoclaved ddH_2O (work under the clean bench)
- the white precipitate will disappear upon addition of Stock F (Step 3)

3 Addition of stocks after autoclaving

Stock D	-	1 mL
Stock F	-	**4 mL**

→ work under sterile conditions (clean bench)

- Stock D contains Fe(III), which tends to precipitate during autoclaving
- Stock F contains HCO_3^-, which vaporizes under heat treatment

- Media can be stored at **room temperature**

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