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

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Protocol status: Working

We use this protocol and it's working

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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
- NaNO₃ as sole (major) nitrogen source (no additional KNO₃)
- 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

1 Prepare the following 8 stock solutions

	Stock	Components	Concentration	Stock concentration (g/L)
	Stock A	K ₂ HPO ₄	4 M	696.8
	Stock B	MgSO ₄ + 7xH ₂ O	2 M	493.0
	Stock C	CaCl ₂ + 2xH ₂ O	500 mM	73.5
	Stock D	Fe-ammonium citrate (Ammonium iron(III) citrate)	150 mM	39.3
	Stock E	NaNO ₃	5 M	425
	Stock F	KHCO ₃	2.5 M	250.4
	EDTA	Na ₂ EDTA	150 mM	55.8
	Trace elements	H ₃ BO ₃	25 mM	1.55
		MnCl ₂ + 4xH ₂ O	20 mM	4.0
		ZnSO ₄ +	2 mM	0.58



		2xH 2O		
		Na ₂ MoO 4 + 2xH 2O	3 mM	0.73
		CuS O ₄ + 5xH 2O	20 μM	0.00 05
		Co(NO ₃) ₂ + 5xH 2O	60 μM	0.01 6

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

Note

If you are working with a promoter that is induced by metals (e.g. Zn²⁺, Cu²⁺, Co²⁺), 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 Cu²⁺ and Co²⁺ in the CD medium!

2 Preparation of 1 L CD medium

- fill ~500 mL H₂O into an autoclavable 1 L bottle
→ do not mix the stock solution without excess H₂O 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 ddH₂O to a final volume of 1 L



- autoclave according to your standard procedure

Note

- autoclaving leads to a significant loss of media volume!
⇒ Fill up the media with autoclaved ddH₂O (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)

Note

- Stock D contains Fe(III), which tends to precipitate during autoclaving
- Stock F contains HCO₃⁻, which vaporizes under heat treatment

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