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High Density Cultivation of *Synechocystis* sp. PCC 6803 using the HDC 6.10B system (CellDeg) V.2

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1 Works for me dx.doi.org/10.17504/protocols.io.9cgh2tw

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ABSTRACT

The CellDeg© high density cultivation system is a revolutionary culturing system for photoautotrophic microorganisms. The culturing vessels come with a semi-permeable membrane on the bottom that allows the diffusion of CO_2, while a membrane in the lid (on top of the vessel) facilitated the passage of O_2. By placing the vessels on top of a highly concentrated carbonate buffer reservoir with high CO_2 partial pressure - in combination with constant agitation, nutrient-rich media and high light intesities - the system allows the obtainment of precedingly unparalleled cell densities [1].

This protocol has been established in the Lindberg lab at Ångström laboratory (Uppsala University) for highly efficient sesquiterpenoid production with *Synechocystis* sp. PCC 6803 using a dodecane overlay as *in situ* extractant.
The protocol has been proven successful for small-scale screening procedures over time periods of up to one week.

[1] Bähr, L., Wüstenberg, A. & Ehwald, R. J Appl Phycol (2016) 28: 783. https://doi.org/10.1007/s10811-015-0614-5

EXTERNAL LINK

http://celldeg.com

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.

MATERIALS

NAME	CATALOG #	VENDOR
Potassium carbonate	View	P212121
Potassium bicarbonate	237205	Sigma – Aldrich

MATERIALS TEXT

HDC 6.10B Starter Kit (CellDeg), CD media (dx.doi.org/10.17504/protocols.io.2bxgapn)

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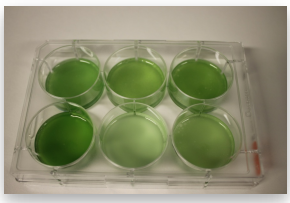
Preparation of precultures

1 **Example: 6 well plate precultures**



- prepare standard polystyrene 6 well plates
  → each 3 wells per strain should be sufficient to inoculate 3 replicates in the Celldeg system
- inoculate 3 mL standard BG11 medium (dx.doi.org/10.17504/protocols.io.wj5fcq6)
  with strains of *Synechocystis* sp. PCC 6803 **3 ml**
- if metal induction (e.g. Cu^{2+} and/or Co^{2+}) is required in the experiment use BG11 w/ modified trace metal composition → *yes, they grow fine without these two trace elements* :-)
- don't forget to add the appropriate antibiotics
- place the 6 well plates on a standard orbital shaker, e.g.:
  'Standard analog shaker, Model 5000 (VWR; orbit: 25 mm; frequency: 120 rpm)
- incubate at **30 °C** under constant light intensities of **50-100 µmol photons · m^{-2} · s^{-1}** **30 °C**
- **after ~ 4 days go to Step 2**

Inoculation of Celldeg cultures

2 **Prepare experimental cultures from precultures**

- measure OD_{750} in a spectrophotometer (e.g. plate reader)
- calculate the volume needed for inoculation of **8 mL Celldeg culture**:

Example:

- measured OD_{750} of preculture = 1.2
- desired OD_{750} in Celldeg vessel = 0.3
- desired volume in Celldeg vessel = 8.0 mL

$$V(preculture) = (0.3 * 8.0mL)/1.2 = 2.0mL$$

- centrifuge preculture (e.g. 2.0 mL) for 5 min at 2500 g and room temperature **2500 x g**
- resuspend pellets in **8 mL CD medium** (dx.doi.org/10.17504/protocols.io.2bxgapn)
  including appropriate antibiotics **8 ml**

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- if desired, supply cultures with inducer molecule
  → note that **higher inducer concentrations** might be required under HD conditions:

Example:

- for Cu^{2+}-mediated induction of the P_{petE} promoter: add **4 µM $CuSO_4$** every second day
- for Co^{2+}-mediated induction of the P_{coaT} promoter: add **30 µM $CoCl_2$** every day

- transfer cell culture to **CellDeg vessel**
- add **2 mL of dodecane** to the cultures (only if *in situ* extraction is desired) 2 ml

Cultivation vessel with dodecane overlay

The CellDeg vials, since CO_2 is provided from the bottom, do not need a large headspace for gas exchange, and can be filled generously (the ~25 mL vials can easily accommodate 10 mL cultures). If no dodecane overlay is added, 10 mL culture volume should be used to quantitatively minimize evaporation effects.

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3 **Bicarbonate-carbonate buffer (reservoir preparation)**

Ingredient	Concentration (M)	Concentration (g/L)
KHCO3	3	270.31
K2CO3	3	41.41

Ingredients of CellDeg carbonate buffer reservoir for a CO_2 partial pressure of **90 mbar at 20°C**

- dissolve $KHCO_3$ 900 mL H_20 **900 ml**
- dissolve K_2CO_3 100 mL H_20 **100 ml**

- due to the high final concentrations, gentle heating (**≤ 40 °C**) of the solutions can accelerate the complete dissolution of the salts (in particular $KHCO_3$)

- you can easily up- or downscale the buffer amounts

  → the desired **mixing ratio** for **3M $KHCO_3$: 3M K_2CO_3 is 9:1**

- due to the high final concentrations, heating of the solution can accelerate the complete dissolution of the salts

Make sure that the vessel is tightly closed before you stir and heat

- as the solution doesn't get in contact with the cultures, it doesn't need to be sterilized

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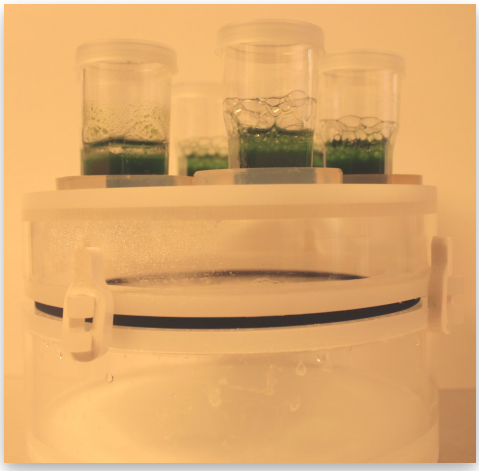
4 Vessel kit assembly

- depending on the size of the CellDeg system, the reservoir container has to be filled by 20% of the total volume by the concentrated carbonate solution
  → fill a standard reservoir with **200 mL bicarbonate-carbonate buffer from step 3**

- attach the **filled vessels from Step 2** to the tray on top of the reservoir



Celldeg Cultivation

5 Cyanobacteria culturing

- place the assembled CellDeg system on an orbital shaker, e.g: IKA KS 130 basic orbital shaker (orbit ø = 4 mm)
  → shake at **320 rpm**

- Incubation Chamber: *Versatile Environmental Test Chamber* (Sanyo) w/o humidifier
  → note that the light sources are laterally aligned

- temperature: **30 °C** 30 °C

- sequence of increasing light intensities: **250 µmol photons * m^{-2} * s^{-1}** (0h-24h),
  490 µmol photons * m^{-2} * s^{-1} (24h-48h), **750 µmol photons * m^{-2} * s^{-1}** (tp 48h-xh)

  → light intensities were measured with a Licor LI-185B quantum meter
  → the values are sums of multi-directional measurements

Note:

The incubation chamber used here is not optimized for this cultivation type and has an upper limit in light intensities. Following the manufacturer's recommendations - particularly regarding the quality of the light source - should distinctly improve the yields.

- the bicarbonate-carbonate buffer in the reservoir should be replaced after 4 days of culturing

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