Preparing cryo cultures (from fresh liquid cultures) of Synechocystis sp. PCC 6803

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Abstract

This protocol describes the preparation of cryogenic cultures from liquid cultures of *Synechocystis* sp. PCC 6803 for a further storage under -80 °C.

The protocol was handed over by Anna Behle MSc.

You can also check the recipe for cyanobacteria in general in 'Synthetic Biology in Cyanobacteria Engineering and Analyzing Novel Functions' by Heidorn *et al.* They use a DMSO concentration of 5% in the final culture and cells that are in late log to early stationary phase. They also use glycerol as a cryptoprotectant.

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Before start

Prepare 0.5 x BG-11 with 8% dimethyl sulfoxide (DMSO).

Inoculate your media with colonies from agar plates, fresh cultures or cryo cultures.

Incubate your liquid cultures for **at least seven days.**

If your cultures contain plasmid with antibiotic restistances, apply appropriate antibiotics in your media.

Protocol

Absorption measurement

Step 1.

Measure the absorption of your samples under the OD750 via photometry.

When your absorption equals 1.0, go to the next step.

Centrifugation

Step 2.

Centrifuge your samples under **4,500 rpm** for **5 minutes**.

Discarding

Step 3.

Discard the supernatant.

Resuspending

Step 4.

Resuspend your pallet in **500µl 1 x BG11 with 8% DMSO** and pipette your samples in sterile cryo culture tubes.

Freezing

Step 5.

Cool your samples with **dry ice or liquid nitrogen**. Quickly store your samples under **-80 °C** for a further storage.

Warnings

Work under **sterile conditions** to **avoid fungal contamination**. Don't freeze yourself while cooling your samples with liquid nitrogen or dry ice.

Make sure to **check your reagents and chemicals for safety warnings and correct waste disposal!**