Jul 12, 2019

Cell lysis and extraction of total protein from *Synechocystis sp*. PCC 6803

Anna Behle[1]

[1]Institute for Synthetic Microbiology

1 Works for me dx.doi.org/10.17504/protocols.io.ps6dnhe

Axmann Lab CyanoWorld view 1 more group

Anna Behle
Institute for Synthetic Microbiology

ABSTRACT

Cell lysis and extraction of total protein. Soluble and insoluble fractions can be further separated. Total protein can be then used for quantification and Western Blot analysis.

BEFORE STARTING

Prepare fresh thylakoid buffer (1x TP).

Before starting

1 Prepare 1x TB (thylakoid-buffer).

Component	Molecular weight (g/mol)	Concentration
HEPES / NaOH, pH = 7	238,3	50 mM
$MgCl_2$		5 mM
$CaCl_2$		25 mM
(optional: glycerol)		*10 % (v/v)*

To 10 mL 1x TB: Add 1 tablet protease-inhibitor (complete ULTRA Tablets, Mini, EDTA-free, EASYpack by Roche)

Culturing

2 Grow cyanobacteria to an OD_{750} of 0.5 - 1.0.

Sampling

3 Sample 10-20 mL of cyanobacterial culture.
Pellet the cells at 4500 rpm, 4 ˚C.

Cell lysis

4 Work on ice.
Resuspend cell pellet in 200 µL 1x TP. Add an equal volume of glass beads (0.1 mm, 0.25 mm 1:1)
Disrupt cells in Precellys homogenizer.
Program: 4000 rpm, 2x 30 sec, 15sec pause, then back on ice

Make sure to use tubes suited for disruption in homogenizer - some tubes will break and you may lose your sample!

5 Centrifuge (4 ˚C, 4000 rpm, 5 min), transfer supernatant to a fresh tube.
Wash glass beads by adding 200 µL 1x TP. Repeat cell lysis step in Precellys.

6 Centrifuge (4 ˚C, 4000 rpm, 5 min), add supernatant to the supernatant from step 5.

Separation of membrane fraction from soluble protein

7 Centrifuge extract at maximum speed for 30 min to 1 h, 4 ˚C.
Soluble protein supernatant will appear blue, membrane pellet green.
Check absorption spectrum of supernatant (chlorophyll peak at 665 nm should be completely gone).