Oct 14, 2019

Disruption of Synechocystis cells

DOI
dx.doi.org/10.17504/protocols.io.76zhrf6

Igem Dusseldorf[1]

[1]Heinrich-Heine Universität Düsseldorf

Igem Dusseldorf

DOI: https://dx.doi.org/10.17504/protocols.io.76zhrf6

Protocol Citation: Igem Dusseldorf 2019. Disruption of Synechocystis cells. **protocols.io** **https://dx.doi.org/10.17504/protocols.io.76zhrf6**

Protocol status: Working
We use this protocol and it's working

Created: October 14, 2019

Last Modified: October 14, 2019

Protocol Integer ID: 28601

Keywords: disrupting synechocystis cell, disruption of synechocystis cells protocol, synechocystis cells protocol, synechocystis cell, steps on ice thylacoid, tablet protease inhibitor, cell disruption, cellular waste from protein, ice thylacoid, ml cell culture, mix whole cell, cells at room temperature, protein until use, ml cell culture of od750, cellular waste, soluble protein, cell, roche complete ultra tablet, whole cell, complete ultra tablet

Abstract

Protocol for disrupting Synechocystis cells to get soluble and non-soluble protein in different fractions

- Carry out all steps on ice
- Thylacoid-buffer (TP)
- 50 mM HEPES/NaOH
- 5 mM $MgCl_2$
- 50 mM EDTA. pH 8
- 1 Tablet Protease inhibitor (Roche complete ULTRA tablets) per 10 ml TP

Cell disruption
Add 200 µl TP to a pellet of 20 ml Cell culture of OD_{750} ~0,5 and resuspend the pellet
Add ca. 200 µl glas beads (1:1 mix of 0,1mm beads and 0,25 mm beads)
Use program 2 of the precellys to disrupt the cells at room temperature, put on ice immediately afterwards
Centrifuge for 5 min at 4°C and 4000 rpm to seperate glas beads and cellular waste from protein
Take supernatant (Whole cell extract 1)
Wash glas beads with 200 µl TP and repeat Precellys disruption
Centrifuge for 5 min at 4°C and 4000 rpm to separate glas beads and cellular waste from protein
Take supernatant (Whole cell extract 2)
Mix Whole cell extract 1 and 2
Optional: Centrifuge for 45 min at 21000 rpm and 4°C to seperate soluble from non-soluble protein. Soluble protein will contain Phycocyanin, non-soluble protien will contain Chlorophyll.
Freeze the protein until use, keep on ice when using it.

Troubleshooting