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Disruption of Synechocystis cells

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Igem Dusseldorf¹

¹Heinrich-Heine Universität Düsseldorf



Igem Dusseldorf

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Protocol status: Working

We use this protocol and it's working

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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 MgCl₂
 - 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 OD₇₅₀ ~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

