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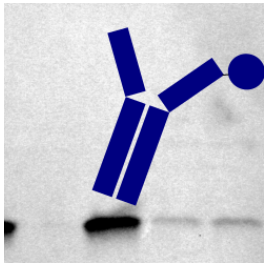
Western blotting in Chlamydomonas reinhardtii V.2



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We use this protocol and it's working

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Abstract

This protocols describe the steps to perform a western blot in Chlamydomonas reinhardtii cell lysate and supernatant samples.

Guidelines

Prepare cell material by cultivating in liquid media.

Before start

- Check antibody dilutions
- Check buffers disponibility
- Prepare 5% milk solution

Sample preparation - Supernatant

1

1. Centrifuge algae culture at 2000xg for 10 min
2. Recover supernatant

00:10:00 Centrifugation time

Sample preparation - Lysate

2

1. Centrifuge algae culture at 2000xg for 10 min
2. Remove supernatant, and re-suspend cells in lysis buffer (50 mM Tris·HCL (pH 8.0), 0.1% Triton X-100), concentrating cells 100-fold.

00:10:00 Centrifugation time

Sonication

3

1. Sonicate using appropriate sonication tip. Duty cycle: 0.5 | Cycle: 1.0 s | Amplitude: 20% | Duration: 30 s
2. Centrifuge for 15 min at 20000xg to remove cells debries and recover soluble proteins
3. Quantificate soluble protein

00:15:00 Centrifugation time

Gel electrophoresis | SDS-PAGE

4

1. Load 30 µg of total soluble protein (TSP) per lane in a 12% SDS-PAGE
2. Transfer proteins to a nitrocelulose membrane
3. Block the membrane with 5% milk solution
4. Probe the desired protein with the specific antibodie, diluted in 5% milk solution
5. Wash 2 times with TBST (0.2 M Tris, 1.37 M NaCl, 0.1% Tween-20, pH 7.6)
6. Add secondary antibody if required

30 µg total solube protein per lane