



Apr 11, 2022

Western blotting in Chlamydomonas reinhardtii V.2

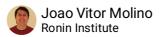
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1



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

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Prepare cell material by cultivating in liquid media.

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- 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

- - 2. Centrifuge for 15 min at 20000xg to remove cells debries and recover soluble proteins
 - 3. Quantificate soluble protein
 - © 00:15:00 Centrifugation time

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2

Gel electrophoresis | SDS-PAGE

- 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

