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Western blotting in *Chlamydomonas reinhardtii* V.2

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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. 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 debris and recover soluble proteins
3. Quantificate soluble protein

🕒 **00:15:00 Centrifugation time**

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 1. Load 30 µg of total soluble protein (TSP) per lane in a 12% SDS-PAGE
 2. Transfer proteins to a nitrocellulose membrane
 3. Block the membrane with 5% milk solution
 4. Probe the desired protein with the specific antibody, 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 soluble protein per lane**