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Protein Extraction and Western Blotting for Sulfolobus

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Abstract

This protocol describes the step-by-step methodology for extracting proteins from cells and analysing them via Western blot. The workflow includes cell lysis, protein quantification, SDS-PAGE electrophoresis, protein transfer to nitrocellulose, antibody incubation, and visualisation using Licor technology. The protocol ensures reproducible results and is adaptable for whole proteome analysis. Alternative lysis buffers, such as RIPA or TK150, may be used depending on downstream applications. The method enables efficient protein detection and quantification, critical for studying protein expression and modifications.

Background

Western blotting is a widely used technique for detecting specific proteins within a complex biological sample. It combines electrophoretic separation, membrane transfer, and antibody-based detection. Compared to alternative methods like ELISA or mass spectrometry, Western blotting offers a balance between specificity, sensitivity, and cost-effectiveness. However, it requires careful handling to avoid protein degradation and non-specific binding. This protocol provides a tailored approach for *Suloflobus acidocaldarius.*

Materials

Product name	Brand	Manufacturer	Catalogue number	Notes
TK150 Buffer (25 mM Tris-HCl, pH 7.4, 2.5 mM MgCl2)	Custom	N/A	N/A	Prepare in-lab
RIPA Buffer	Thermo Scientific	Thermo Fisher Scientific	89900	For whole proteome analysis
Triton X-100	Sigma-Aldrich	Merck	X100	Non-ionic surfactant
Benzonase/DNase I	Merck	Merck	70746	Nuclease for DNA removal

Product name	Brand	Manufacturer	Catalogue number	Notes
EDTA-free Protease Inhibitor Cocktail	Pierce	Thermo Fisher Scientific	78425	Prevents protease activity
Bradford Reagent	Bio-Rad	Bio-Rad Laboratories	5000006	For protein quantification
SDS Loading Buffer	Laemmli Sample Buffer	Bio-Rad Laboratories	1610737	Contains SDS for sample preparation
MES Buffer	Thermo Scientific	Thermo Fisher Scientific	B0002	Running buffer for SDS-PAGE
Molecular Weight Marker (Ladder)	Precision Plus	Bio-Rad Laboratories	1610374	Protein standard
5x Transfer Buffer (15.1g Tris Base, 94g Glycine in 1L ddH2O)	Custom	N/A	N/A	Dilute to 1x with methanol before use
Ponceau Stain	Sigma-Aldrich	Merck	P7170	Reversible protein stain
0.2% PBS-Tween with Milk Powder	Custom	N/A	N/A	Blocking solution
Primary Antibodies	Various	Various	Various	Depending on target protein
Licor Secondary Antibodies	IRDye	LI-COR Biosciences	926-32211	Fluorescent secondary antibodies
Filter Paper	Whatman	Cytiva	3030-917	For blotting
Nitrocellulose Membrane	Protran	Cytiva	10600002	Protein transfer membrane
Pre-cast Gels	Mini-PROTEAN TGX	Bio-Rad Laboratories	4561096	SDS-PAGE gels
Deionized Water (ddH2O)	N/A	N/A	N/A	For reagent preparation
Ice	N/A	N/A	N/A	To maintain sample temperature

Equipment

Equipment name	Brand	Manufacturer	Catalogue number	Notes
Sonicator	Bioruptor	Diagenode	UCD-200	Ultrasonic cell disruptor
Centrifuge (4°C)	Eppendorf	Eppendorf	5404000538	Refrigerated microcentrifuge
Heat Block (99°C)	ThermoMixer	Eppendorf	5382000023	For sample incubation
Spectrophotometer	NanoDrop	Thermo Fisher Scientific	ND-2000	Nucleic acid and protein quantitation
Transfer Chamber and Sponges	Trans-Blot SD	Bio-Rad Laboratories	1703940	Semi-dry transfer system
Gel Electrophoresis Chamber	Mini-PROTEAN Tetra	Bio-Rad Laboratories	1658004	For running SDS-PAGE gels
LI-COR Imaging System	Odyssey CLx	LI-COR Biosciences	9140-01	For fluorescent Western blot detection

Protocol

Protein Extraction and Quantification

Note: Keep samples and cell pellets on ice at all times.

1. Resuspend cell pellets (from 25 mL of culture) in 250 µL TK150 buffer with:
    - Benzonase/DNase 1 (1:1000 dilution)
    - EDTA-free protease inhibitor cocktail (1:1000 dilution)
    - 0.1% Triton X-100 (Vortex to mix thoroughly)

2. Disrupt the cells using a cup sonicator:
    - Set the sonicator to 4°C.
    - Place samples in Bioruptor microtubes.
    - Sonicate for 30 seconds, followed by 30 seconds rest, for 10 cycles.
    - Centrifuge at maximum speed for 15 minutes at 4°C.

3. Quantify protein concentration using the Bradford assay:
    - Mix 800 µL ddH2O and 200 µL Bradford reagent.
    - Prepare one cuvette per sample, plus one blank (5 µL TK150 buffer mix).
    - Add 5 µL of protein supernatant to each cuvette, mix, and measure OD595.
    - Use an albumin standard curve for quantification.

Western Blot

4. Load and Run SDS-PAGE Electrophoresis:
    - Mix 5 µg of protein per sample with SDS loading buffer.
    - Boil samples at 99°C for 2 minutes.
    - Load samples into pre-cast gel wells.
    - Add 3 µL molecular weight marker.
    - Run at 80V for stacking gel and 120V for resolving gel in MES buffer.
    - Stop when dye markers reach the bottom; remove gel.

5. Protein Transfer to Nitrocellulose:
    - Prepare 1x transfer buffer (add methanol).
    - Wet sponges, filter paper, and nitrocellulose.
    - Assemble the transfer sandwich: Black sponge → Filter paper → Gel → Nitrocellulose → Filter paper → White sponge
    - Place in transfer chamber with ice block.
    - Run for 1 hour at 100V.

6. Stain and Scan Nitrocellulose:
    - Rinse membrane with ddH2O.
    - Stain with Ponceau and wash with 5% acetic acid.

- Scan using 'refractive' and 'positive film' settings.

7. Blocking and Antibody Incubation:
    - Block membrane in 5% milk-PBST (0.2% Tween) for 1 hour while rocking.
    - Incubate overnight at 4°C in 10 mL milk-PBST with primary antibody.

8. Washing and Secondary Antibody Incubation:
    - Wash membrane 3 times, 15 minutes each, in PBST.
    - Incubate in 10 mL milk-PBST with Licor secondary antibody for 1 hour.
    - Wash again 3 times in PBST.
9. Develop Using Licor Imaging System:
    - Place membranes inside a dark plastic folder.
    - Scan using Licor machine.

Data analysis

Analyse band intensities using Image Studio (Licor) or other quantification software. Normalize protein levels to a loading control (e.g., GAPDH, β-actin).

Recipes

5x Transfer Buffer:

- 15.1g Tris Base
- 94g Glycine
- Dissolve in 1L ddH2O

Additional Notes

- Maintain samples on ice to prevent protein degradation.
- Take care to remove all bubbles during the transfer step.
- Always use freshly prepared blocking solutions.

Competing interests

The authors declare that they have no conflict of interest.

Acknowledgments

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