Jun 04, 2025

Western Blot

In 2 collections

DOI
https://dx.doi.org/10.17504/protocols.io.eq2lyqknrvx9/v1

Lucas Blasco-Agell[1,2], Jara Montero-Muñoz[1,2], Meritxell Pons-Espinal[1,2], Antonella Consiglio[1,2], Miquel Vila[3]

[1]Department of Pathology and Experimental Therapeutics, Bellvitge University Hospital- IDIBELL, 08908 Hospitalet de Llobregat, Spain;
[2]Institute of Biomedicine of the University of Barcelona (IBUB), Carrer Baldiri Reixac 15-21, Barcelona 08028, Spain;
[3]VHIR-CIBERNED-ASAP

Vilalab Public

Miquel Vila
VHIR-CIBERNED-ASAP

DOI: https://dx.doi.org/10.17504/protocols.io.eq2lyqknrvx9/v1

Protocol Citation: Lucas Blasco-Agell, Jara Montero-Muñoz, Meritxell Pons-Espinal, Antonella Consiglio, Miquel Vila 2025. Western Blot. **protocols.io https://dx.doi.org/10.17504/protocols.io.eq2lyqknrvx9/v1**

Protocol status: Working
We use this protocol and it's working

Created: June 04, 2025

Last Modified: June 04, 2025

Protocol Integer ID: 219571

Keywords: western blot

Funders Acknowledgements:
ASAP
Grant ID: ASAP-020505

Abstract

Western Blot

Troubleshooting

1 For protein isolation, cell pellets are resuspended in RIPA buffer with 1X protease inhibitors, sonicated, and centrifuged at 10,400x*g* for 10 minutes at 4°C.

2 Supernatant is collected and protein concentration is determined by Bradford assay.

3 Protein is mixed with 6x LSB (Sigma-Aldrich) and 4% DTT (Sigma-Aldrich) and heated at 95 ºC for 5 minutes in a Thermomixer.

4 Samples are subjected to SDS-PAGE on 8 to 12% of polyacrylamide and samples are run with a PowerPac Basic (Bio-Rad Laboratories) at 90-120 Volts for 60-90 minutes in a Mini-Protean® 3 Cell (Bio-Rad Laboratories).

5 Gel is transferred at 350 Amperes for 120 minutes onto pre-activated polyvinylidene difluoride (PVDF) or Nitrocellulose membranes in a Mini-Protean® 3 Cell (BioRad Laboratories). Correct protein transfer is checked by red Ponceau S Solution (Panreac).

6 Membrane is rinsed 3 times with TBS with 0.1% of Tween20 and blocked at RT for 1 hour with 5% milk or 5% BSA and incubated with primary antibody at 4°C ON.

7 The day after, membrane is rinsed and incubated with secondary Horseradish Peroxidase (HRP)-labelled antibody at RT for 1 hour.

8 After rinsing, signal is developed with ECL™ start Western Blotting Detection Reagent (GE Healthcare Amersham™) and images are obtained using a ChemiDoc System (Bio-Rad Laboratories).

9 Protein amount is expressed as a ratio between the band intensity of the protein of interest and the loading control protein β-Actin or Glyceraldehyde-3- Phosphate Dehydrogenase (GAPDH).