Western Blotting

www.davids-bio.com (Custom Antibodies)
www.davids-science.de (Lab Material)

- 1 - Introduction

Western blotting, also known as immunoblotting is a technique to detect specific proteins within a biological sample. It is a powerful tool with various applications, including protein expression analysis, protein-protein interaction studies and protein quantification.

The immunoblotting process involves several steps. First, proteins are separated by size using gel electrophoresis (SDS-PAGE). In a second step, the proteins are transferred from the gel onto a solid membrane. This transfer process is known as blotting and ensures that the protein bands maintain their arrangement. The membrane is treated with blocking solutions and antibodies to detect specific proteins. The resulting bands on the membrane correspond to the presence and size of the target protein in the original sample.

- 2 - Material

Material	
SDS Sample Buffer	125 mM Tris-HCl 4% w/v SDS 20% Glycerol 10% ß-Mercaptoethanol 0.004% Bromphenol Blue pH 6.8
Running Buffer	25 mM Tris 200 mM Glycine 0.1% SDS pH 8.5
Transfer Buffer	25 mM Tris 200 mM Glycine 0 – 15% Methanol 0.1% SDS pH 8.5
Blocking Buffer	1 x TBS 0.1% Tween-20 5% BSA **or** Milk Powder

	Stirr and filtrate	
Wash Buffer	1 x TBS 0.1% Tween-20	
Primary Antibody		
Secondary Antibody	Conjugated anti-species antibodies	www.davids-science.de

- 3 - Method

Sample Preparation

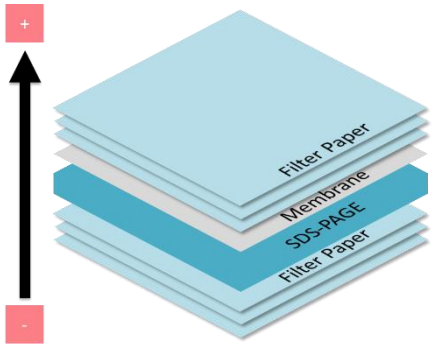
- Use an appropriate amount of sample solution by determining the protein concentration by Bradford or 280 nm
- Dilute the sample with SDS Sample Buffer 1:2 – 1:5
- Heat the sample to 95°C for 5 minutes
- Store the samples at -20°C or continue with the protocol

SDS-Gel

- 10% gels can be used for most proteins in the range of 10 – 100 kDa
- Pipet equal amounts of protein ranging from 10 – 200 ng or cell lysates ranging from 10 – 40 µg onto the gel
- You may want to use pre-stained markers to have a control for membrane blotting
- Run the gel at 140 V until the dye front reached the bottom of the gel
- You may want to start with 80 V and turn it up after 20 minutes

Electrotransfer

- Use Nitrocellulose or PVDF membranes
- PVDF membranes must be wetted with Methanol for 1 minute before usage
- Remove the Methanol with Transfer Buffer
- Follow the instructions of your Blotter or Membrane for optimal results



Staining

- Block the membrane with Blocking Solution for 1 hour at room temperature
- You may want to block the membrane over night at 2 – 8°C
- Rinse the membrane three times with Wash Buffer
- Incubate the membrane with your primary antibody over night at 2 – 8°C
  Affinity purified antibodies: 0.1 – 20 µg/ml (e.g., 1:100 from a 1.0 mg/ml antibody solution)
  ProteinA purified antibodies: 1 – 200 µg/ml (e.g., 1:10 from a 1.0 mg/ml antibody solution)
- Rinse three times with Wash Buffer
- Incubate the membrane with your secondary antibody for 1 hour at room temperature
  Please refer to the manual of the secondary antibody for recommended dilution
- Rinse three times with Wash Buffer
- Incubate the membrane with your substrate of choice
  Please refer to the manual of the secondary antibody or substrate (e.g., BCIP/NBT, DAB)

- 4 - Trouble Shooting

Reason	Solution
Secondary Antibody	Use a secondary antibody that is specific to the host species For example, use goat anti-rabbit HRP antibodies for polyclonal rabbit antibodies Sodium Azide can inhibit HRP from secondary antibody You may want to use a secondary antibody that detects IgG and IgM antibodies
Primary Antibody Concentration	The dilution of the primary antibody might be too high. We recommend to try up to 20 µg/ml for antigen-specific antibodies Avoid freezing/thawing cycles of the primary antibody Most antibodies can be stored at 2 – 8°C for months
Analyte/Protein Concentration	Increase the amount of lysate or protein for a better detection. Ensure you have the correct analyte concentration by Bradford and use fresh analytes
SDS-PAGE	Check with a protein ladder and the size of your analyte that the analyte does not run off the gel
Transferring	You can check the transfer success with ponceau red
Incubation Time	To receive a higher signal, incubate the primary antibody over night at 2 – 8°C
Blocking	Decrease incubation time to 1 h at room temperature You may want to try IgG and lipid free BSA instead of Milk Powder Add 0.1 – 0.6 % Tween 20 in blocking buffer to reduce cross reaction of the antibody with BSA and Milk Powder
Membrane	For PVDF, please activate the membrane before use with Methanol Nitrocellulose usually binds less analytes and has less background compared to PVDF
Substrate	Use fresh substrate for the enzyme reaction