DAVIDS PROTOCOLS

Davids Biotechnologie

WesternBlot

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Material

SDS Sample Buffer	Running Buffer	Transfer Buffer (wet)	Blocking Buffer
125 mM Tris-HCl 4% w/v SDS 20% Glycerol 10% β-Mercaptoethanol 0.004 % Bromphenol Blue pH 6.8	25 mM Tris base 200 mM Glycine 0.1% SDS pH 8.5	25 mM Tris base 200 mM Glycine 0 - 15% Methanol 0.1% SDS pH 8.5	1 x TBS 0.1% Tween-20 5% BSA **or** Milk Powder Stirr and filtrate

Wash Buffer
1 x TBS 0.1% Tween-20

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 to 1:5
- Heat the sample for 95°C for 5 min
- 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 (10 – 200 ng) or cell lysates (10 – 40 µg) to the gel
- You may want to use pre-stained markers to have a control for membrane blotting
- Run the gel: 80 – 140 V for 1 – 2 hours. You may want to start with 80 V and turn it up after 10 – 20 min

Electrotransfer to a Membrane

- Use Nitrocellulose or PVDF. PVDF must be wetted with Methanol for 1 min before usage. Remove the Methanol with Transfer Buffer.
- You may want to dye the membrane with Ponceau red to check if the transfer is successful
- Follow the instructions of your Blotter or Membrane for optimal results

Staining with Antibodies

- **Blocking:** 1 h at room temperature with blocking solution (Alternatively block at 2 – 8°C over night).
- **Wash:** Membrane 3 times with Wash Buffer
- **Primary Antibody:** Incubate the membrane with your primary antibody over night at 2 – 8°C
  Affinity purified antibodies: 0.1 – 20 µg/ml (i. e. 1:50 from a 0.5 mg/ml antibody solution)
  ProteinA purified antibodies: 1 – 200 µg/ml (i. e. 1:100 from a 10 mg/ml antibody solution)
- **Wash:** Membrane 3 times with Wash Buffer
- **Secondary Antibody:** Incubate 1 h at room temperature with the recommended dilution of the antibody (refer to manual of the secondary antibody)
- **Wash:** Membrane 3 times with Wash Buffer
- **Substrate:** Follow the instructions of your secondary antibody or substrate (i. e. BCIP/NBT, DAB)

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No Signal

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. Try to avoid freezing/thawing cycles of the primary antibody. Use aliquots and thaw each aliquot only once. 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 Problems	Check with a protein ladder and the size of your analyte that the analyte does not run off the gel.
Transferring Problems	You can check the transfer success with ponceau red.
Incubation Time	Try to incubate the primary antibody over night at 2 – 8°C.
Blocking Problems	You may want to decrease incubation time to 1 h at room temperature. You may want to try IgG free BSA instead of Milk Powder.
Membrane	When you use PVDF please activate the membrane with Methanol. Nitrocellulose usually binds less analytes and has less background compared to PVDF.
Substrate	Use fresh substrate for the enzyme reaction.

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High Background

Reason	Solution
Analyte Concentration	Reduce the amount of analyte
Antibody Concentration	Reduce the concentration of the primary and secondary antibody.
Incubation of primary antibody	Incubate the primary antibody over night at 2 – 8°C
Blocking Solution	Add 0.1 – 0.6 % Tween 20 in blocking buffer to reduce cross reaction of the antibody with BSA and Milk Powder. You may want to use IgG free and lipid free BSA instead of milk powder
Blocking Problems	Increase washing time and add Tween 20 to the blocking buffer
Membrane	You may want to use Nitrocellulose for less background compared to PVDF
Substrate	Reduce incubation time of the substrate. Reduce Substrate concentration.
Membrane Handling	Don't touch the membrane to avoid high background.