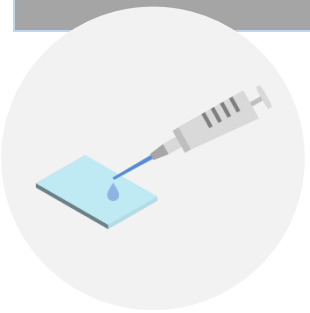


Dot Blotting

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

- 1 - Introduction

The DotBlot is a simple and rapid technique used to detect specific proteins, nucleic acids, or other molecules immobilized on a membrane. Unlike Western blotting, the Dot Blot does not require electrophoresis, making it more straightforward and faster. In a Dot Blot, samples are directly applied as spots onto a membrane, followed by probing with specific antibodies to detect the target molecule. This method is particularly useful for screening multiple samples or when protein concentration is more important than molecular size information.

The DotBlot can be used for protein and antibody detection, semi-quantitative analysis of protein concentrations as well as for the screening of antibody binding specificity. This protocol is for the detection of proteins blotted on a membrane.

- 2 - Material

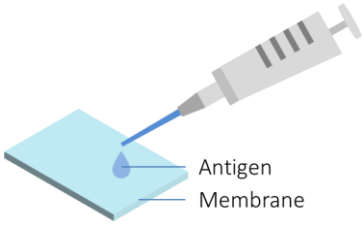
Material		
Membrane	Nitrocellulose or PVDF	We recommend Nitrocellulose
Blocking Buffer	1 x TBS 0.1% Tween-20 5% BSA **or** Milk Powder Stirr and filtrate	
Wash Buffer	1 x TBS 0.1% Tween-20	
Primary Antibody	Get your custom antibody directly from Davids	www.davids-bio.com
Secondary Antibody	Conjugated anti-species antibodies We recommend alkaline phosphatase in combination with NBT/BCIP substrates	www.davids-science.de

- 3 - Method

Preparation of the membrane

- Cut a piece of nitrocellulose or PVDF membrane to the desired size
- When PVDF is used, pre-wet the membrane with methanol for about 10 seconds, then rinse in water and equilibrate in PBS or TBS.

Sample Application



- Dilute your protein or sample to the desired concentration. Typically, 1 – 5 µL of sample is sufficient. It is recommended to pipet only 0.5 – 1 µl at once, let it dry and add the same amount on the dried spot multiple times, until the whole amount is reached.
- Spot the sample directly onto the membrane using a pipette. Allow the spots to air dry for about 10 – 15 minutes.

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, TMB)

- 4 - Trouble Shooting

Reason	Solution
Secondary Antibody	Use a secondary antibody that is specific to the host species Sodium Azide can inhibit HRP from secondary antibody
Primary Antibody Concentration	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
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
Weak or no signal	Ensure that the sample concentration is sufficient and properly spotted onto the membrane. Verify that the primary antibody is specific to the target and that it is used at the correct dilution. Make sure the blocking step was done thoroughly to reduce background noise.
High Background	Increase the number of washing steps or extend washing times. Try a different blocking agent or adjust the concentration. Reduce the concentration of the primary or secondary antibody, as excessive antibody can cause high background.
Uneven Spots	Ensure that the membrane is completely flat during sample application. Avoid using too much sample volume, which can cause diffusion of the spots. Dry the spots thoroughly before proceeding with the blocking step.
Spots Too Large	Use a smaller volume of the sample or reduce the concentration. Apply the sample gently to avoid spreading it on the membrane.