WesternBlot

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Solutions

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 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. TMB, pNPP)