Western Blot

Transfere:

- Run a SDS gel
- Soak 6 filter papers and a nitrocellulose membrane in your transfere buffer for 15 minutes.
- Stack 3 filter papers, the membrane, the SDS gel and another 3 filter papers in your semi-dry blotter. The nitrocellulose membrane should be nearest to the positive electrode.
- Blot the gel to the membrane according to the specifications of your blotter.
- Mark your Protein Ruler with a pencil, so it will be visible after staining the membrane.

Staining:

- Block the membrane for 1 hour in transfere buffer with 3% BSA.
- Dilute your primary antibody in transfere buffer with 3% BSA and incubate the membrane with it for 1.5 hours.
- Wash the membrane 5 times for 5 minutes each with fresh transfere buffer.
- Dilute your secondary antibody in transfere buffer with 3% BSA and incubate the membrane with it for 1 hour.
- Wash the membrane 5 times for 5 minutes each with fresh transfere buffer.
- For chemiluminescent detection, follow the instructions for the reagent you are using. Acquire image.

http://2016.igem.org/Team:Bielefeld-CeBiTec/Experiments/Protocols