Western blot

Sample preparation

Lysis of sample in appropriate lysis buffer (eg. RIPA).

Protein assay to determine protein concentration

Reduce and denature sample (unless stated otherwise on antibody datasheet).
Add sample buffer (SDS and β mercaptoethanol). Heat 95˚C 5 min.

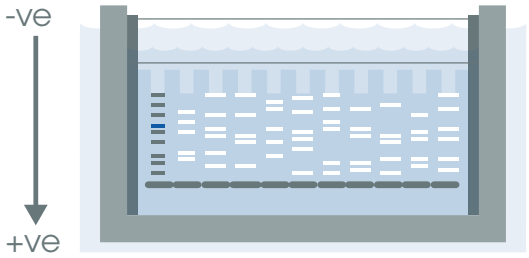
Loading the gel

Optimize lysate amount depending on expression level of the protein.

Prepare running buffer. Assemble the gel in the tank

Running the gel

Smaller proteins (negatively charged) move more quickly through the gel towards the positive cathode.
Proteins separate out according to size.



100 V - 200 V for 30 min to 2 hrs.
Optimize time and voltage.
Follow manufacturers instructions.

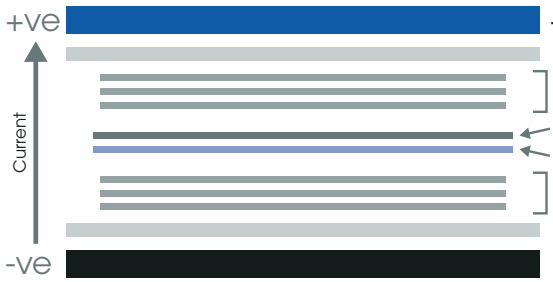
Gel percentage depends on size of protein:

4-40 kDa	20%
12-45 kDa	15%
10-70 kDa	12.5%
15-100 kDa	10%
25-200 kDa	8%

Transfer proteins from the gel to membrane

Prepare transfer buffer.
Cut a piece of membrane.
Transfer the membrane to 1 x transfer buffer.

Assemble transfer stack



Optimize transfer time and voltage depending on the size of your protein. Follow manufacturers instructions.

Negatively charged proteins move up towards the positive cathode and onto the membrane.

Check the transfer.
Ponceau red staining of the membrane or Coomassie staining of the gel.

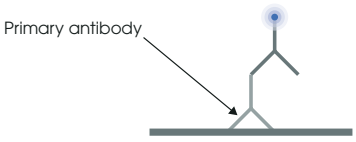
Blocking

Incubate membrane in the appropriate blocking buffer for your antibody i.e. milk or BSA.
Check which blocking buffers have been previously validated for use with your specific antibody.

Primary antibody incubation

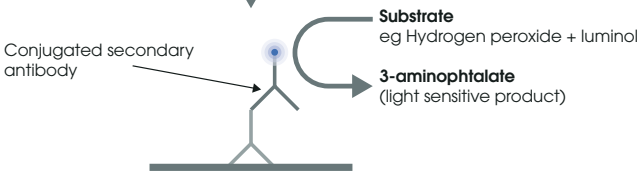
Incubate membrane in primary antibody diluted in blocking buffer for 1–2 hrs RT or 4˚C overnight at the recommended concentration.

Secondary antibody incubation



Incubate with secondary antibody (eg HRP conjugated) diluted in blocking buffer for 1–3 hrs RT at the recommended concentration.

Detection (eg. ECL detection)



Scan and analyze results