Western blot

Sample preparation
Lysis of sample in appropriate lysis buffer (e.g., RIPA).

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

Protein assay to determine protein concentration

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.

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

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%

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

Smaller proteins (negatively charged) move up towards the positive cathode and onto the membrane.

Transfer proteins from the gel to membrane

Assemble transfer stack

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 (e.g., milk or BSA).
Check which blocking buffers have been previously validated for use with your specific antibody.

Primary antibody incubation
Band of protein/antigen on membrane
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 (e.g., HRP conjugated) diluted in blocking buffer for 1-3 hrs RT at the recommended concentration.

Detection (e.g., ECL detection)
Conjugated secondary antibody
Substrate
eg Hydrogen peroxide + luminol
3-aminophthalate
(light sensitive product)

Scan and analyze results

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