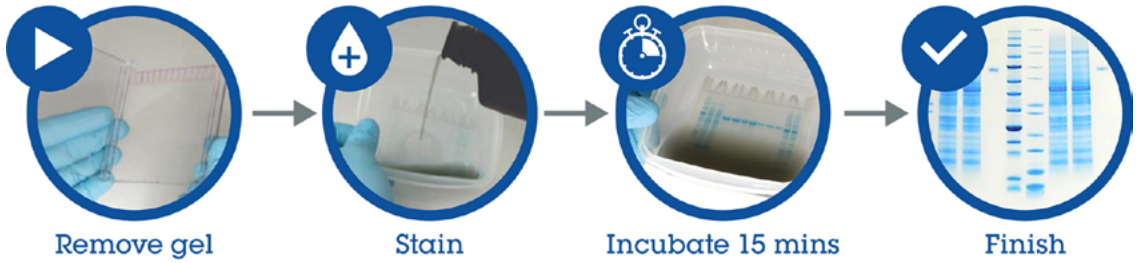
Australian distributors:
Fisher Biotec Australia
free call: 1800 066 077
email: info@fisherbiotec.com
web: www.fisherbiotec.com

One step, ultra fast InstantBlue® Coomassie Protein Stain at Abcam now!



Easy-to-use, sensitive, non-toxic, allowing Coomassie protein staining for polyacrylamide gels with low background in 15 minutes.

Expedeon proteomics and immunology are now part of Abcam. All products can be ordered in Australia through Fisher Biotec. Explore our total solution for WB tools, including below:

SDS PAGE related products and buffers

Product	Cat no.	Size
10X Blocking Buffer	ab126587	50 ml
10X Phosphate Buffered Saline	ab270748	1 pack
20x PBS Buffer with Tween 20	ab64247	125 ml, 1000 ml
20x TBS-T with Tween 20	ab64204	125 ml, 1000 ml
25x TBS (pH 7.4)	ab64248	125 ml, 1000 ml
25x PBS Buffer pH 7.6	ab64026	125 ml, 1000 ml
InstantBlue Coomassie Protein Stain	ab119211	1000 ml
Optiblot SDS Run Buffer (20X)	ab119197	500 ml
Ponceau S Solution	ab270042	1000 ml
SDS Running Buffer - RunBlue™	ab270468	4000 ml
Tris-Glycine SDS Blot Buffer - RunBlue™	ab270227	500 ml, 4000 ml
Tween 20 (Polyoxyethelenesorbitan Monolaurate)	ab128987	125 ml
Western Blot Stripping Buffer	ab270550	500 ml, 5000 ml

ECL and other detection kits

Product	Cat no.	Size
Chemiluminescent Reagent Kit	ab79907	1 kit
ECL Express - LumiBlue™ (20pg)	ab270542	250 ml, 500 ml
ECL Extended - LumiBlue™ (150fg)	ab270494	100 ml, 200 ml
ECL Extra - LumiBlue™ (500fg)	ab270229	250 ml, 500 ml
ECL Extreme - LumiBlue™ (50fg)	ab270517	100 ml, 200 ml
ECL Pico - LumiBlue™ (2pg)	ab270531	250 ml, 500 ml
Fluorescent Western Blot Kit	ab133410	10 tests
His-tagged Protein Gold Detection Kit	ab170734	1 kit

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.

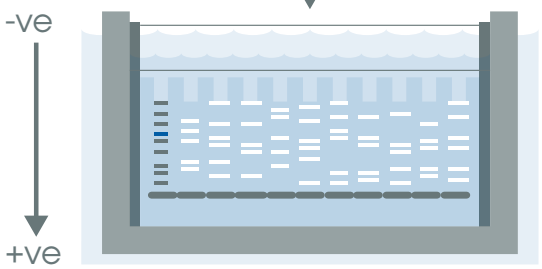
Loading the gel

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.

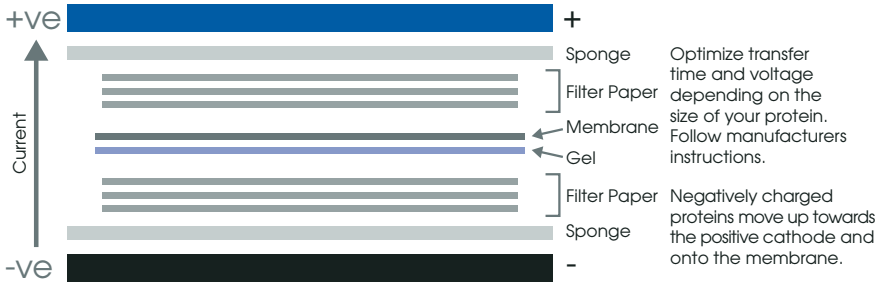
Gel percentage depends on size of protein:

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 (ab119211) of the gel.

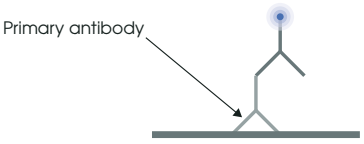
Blocking

Incubate membrane in the appropriate blocking buffer for your antibody i.e. milk or BSA (ab270701).
Check which blocking buffers have been previously validated for use with your specific antibody.

Primary antibody incubation

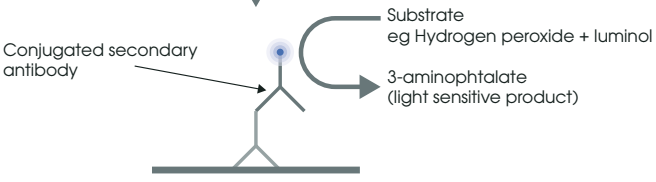
Incubate membrane in primary antibody diluted in blocking buffer for 1–2 hrs RT or 4˚C overnight at the recommended concentration.

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