Western Blot

The Western Blot is an analytical method for the detection of proteins. Primary antibodies bind to specific protein sequences and are detected by a secondary antibody. The secondary antibodies are usually tagged as readout molecules with an enzyme or luminescent substance.

Materials

Filter paper
SDS Gel
SDS sample buffer
Protein Ladder
Protein solution
Membrane
5% milk solution
Prim. antibodies
Sec. antibodies
Falcon

Instruments:

Blot chamber
Pipette
SDS gel chamber
Shaker

Procedure

Prepare SDS gel (protein samples 12µL; marker 3µL)
Run chamber at 110Volt for 1 hour

Prepare blot chamber
Activate membrane in activating solution
Add 4 filter to anode solution I
Add 4 filter to anode solution II
Add 8 filter to cathode solution

Remove gel from chamber
Transfer gel to blot chamber

Blot chamber construction:

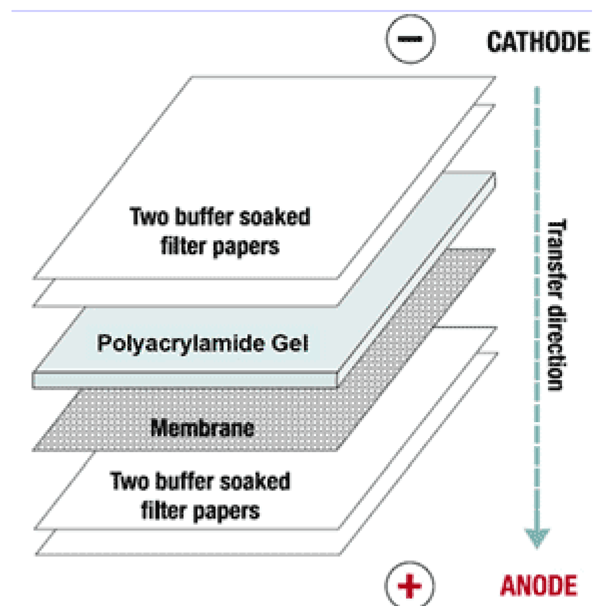

Figure 1: Blot-Sandwich

Push air bubbles out of Blot-Sandwich and close chamber
Run blot chamber at 60mA 1. 1h (with one gel)

Remove the membrane and wash in 5% milk solution (blocking).

Shake for 1h

Fill Falkon 1 with primary antibody solution
Remove membrane and transfer to Falcon 1
Shake Falkon 1 o/n at 5°C.

Fill Falkon 2 with secondary antibody solution
Transfer diaphragm to Falkon 2
Shake Falkon 2 at RT 3h

Remove membrane

Evaluate membrane via the respective readout.

Trouble shooting

There are many different factors that can affect a Western blot. Antibodies are an important factor. A pre-test of these with positive samples should be carried out under all circumstances in order to eliminate possible sources of error.

Bubbles should be avoided when Blot-Sandwiches.

The blot sandwich must not be allowed to dry out as this may destroy the membrane.

Figures

http://technologyinscience.blogspot.com/2011/12/western-blot-protein-immunoblot.html#.W8ZR0WgzaUk