Western Blot Troubleshooting Tips

Western Blot result depends on the whole system including antigen content, sensitivity of primary antibody, sensitivity of secondary antibody, sensitivity of substrate, efficiency of color development and photographic fixing. Mistake of any procedure in the experiment may lead to unsatisfactory results.

Here Elabscience lists the common Western Blot troubleshooting.

1. No band or low band

Possible causes	Suggestions
The loading amount of sample is low	Increase the loading amount
The target protein quantity is too low or not expressed in the sample	Refer to the relevant literatures to make sure that the sample contain your target protein, or prepare fresh sample again and choose a positive sample as control
There is a poor transfer of protein to membrane or not enough protein is bound to the membrane	Ensure that the transfer order is correct and the transfer time is sufficient
The antibody concentration may be too low	Use a higher concentration of antibody
The antibody concentration may be too high to cause the signal to disappear instantly	Use a lower concentration of antibody
Insufficient exposure time	Prolong the exposure time
Insufficient incubation or deactivation of the substrate	Increase the incubation time of substrate and ensure that the substrate is valid
The target protein transferred to the membrane has degraded	Keep a low transfer temperature, decrease the transfer electric current and transfer time
Excessive washing of the membrane	Reduce the frequency or duration of washing steps
The antibody is inactive or the titer of antibody is too low	Pay attention to the preservation of antibody and use antibody with higher titer

2. High background

Possible causes	Suggestions
The experimental equipment has been contaminated	Ensure that the equipment is clean
Some membrane may cause high background	NC membranes are considered to cause less background than PVDF membranes.
Blocking buffer is not compatible or there is cross-reactivity between the blocking buffer with antibodies	Replace the blocking buffer
Insufficient blocking	Prolong the blocking time
The antibody concentration may be too high	Use a lower concentration of antibody
Insufficient washing	Increase the frequency and duration of washing
Excessive exposure time	Shorten the exposure time
The membrane or buffer has been contaminated	Use the fresh buffer and keep the membrane moist during the experiment

3. Non-specific bands

Possible causes	Suggestions
The protein sample has digested during the treatment	Choose fresh samples for experiment
The loading amount of sample is too much	Reduce the loading amount
Insufficient blocking	Prolong the blocking time
The washing of membrane may be insufficient	Ensure sufficient washing of membrane
The antibody concentration may be too high	Use a lower concentration of antibody
The antibody specificity is low	Use antibody with good specificity
The target protein has multiple spliceosomes or modified sites	Refer to relevant literatures to check whether the target protein has other spliceosomes or modified sites

4. Other problems

Problems	Possible causes	Suggestions
Black dots on the membrane	The antibodies may have non-specifically binding with the blocking buffer	Replace the blocking buffer
White bands	The target protein content is too high or antibody concentration is too high	Reduce the loading amount or decrease the concentration of the antibody
Molecular weight is very low or high	Inappropriate gel percentage or uneven gel. /The electrophoresis temperature may be too high	Change the gel percentage: use a higher percentage for small proteins and a lower percentage for large proteins
Uneven bands/ Bands trail or deviate or diffuse to both sides	The equipment is not suitable. / There is bubble at the bottom/ The sample is not dissolved well. / The electrode is not balanced. / The sample amount is too much	Ensure that the electrophoresis gel is in good state and horizontal position/ Ensure the sample extraction/ Reduce the sample amount

Summary:

The Western blot technology is rather mature, but it is not easy to get the desired result with just one trial. It is recommended to explore and optimize the experimental conditions, thus to get your ideal Western bands in the formal experiment.