37 Western Blotting Troubleshooting Tips

ASPIRE
TO CURE

AntibodyGenie

37 WESTERN BLOTTING TROUBLESHOOTING TIPS

NO BANDS OBSERVED

Problems	Explanation
1. Incorrect primary antibody	Antibody has low to no affinity.
2. Inactive antibody	Perform a dot blot.
3. Insufficient protein concentration	Increase the amount of protein and use a positive control.
4. Poor transfer	Make sure the membrane is activated. Transfer buffer must contain methanol when using nitrocellulose membranes. PVDF membranes must be pre-soaked with methanol.
5. Suboptimal transfer time	High molecular weight proteins may require longer transfer times.
6. Incorrect secondary antibody	Confirm host species and IgG type of primary.
7. Antibodies expired	Check that all antibodies are in date.
8. Incorrect storage of antibodies	Ensure all antibodies are stored as per manufacturer's instructions.
9. Sodium Azide contamination	Sodium Azide contamination will quench HRP signal.
10. Suboptimal primary antibody incubation time	Increase incubation time with the primary antibody.
11. Incompatible primary and secondary antibody	Maintain a consistent species in both antibodies.
12. Insufficient secondary antibody concentration	Increase the concentration of primary/secondary antibody.
13. Excessive washing	Reduce the number and duration of washes.
14. Incorrect orientation	Mark your membrane to ensure correct orientation.

POOR QUALITY TRANSFER

Problems	Explanation
15. Membrane choice	Choose either PVDF/nitrocellulose membranes according to the target protein molecular weight.
16. Dry membrane	It is important not to let the membrane or filter paper dry out.
17. Incomplete protein resolution	Ensure optimal gel concentration is used for the protein of interest.
18. Incorrect sample preparation	The sample must contain DTT or B-Mercaptoethanol and be heated prior to loading.

HIGH BACKGROUND

Problems	Explanation
19. Unspecific antibody binding	Ensure the correct and most specific primary antibody is used.
20. Insufficient blocking	Optimise blocking time duration.
21. Suboptimal antibody concentration	Optimise antibody concentration.
22. Insufficient washing	Increase the number of washes performed. Increase the concentration of Tween 20 used in wash buffer.
23. Incorrect membrane choice	Nitrocellulose membranes generally have less background compared to PVDF.
24. Film overexposed	Reduce the exposure time.

TOO MANY BANDS

Problems	Explanation
25. Unspecific antibody	Ensure the antibody used is specific for the protein of interest.
26. Proteolytic breakdown	Use protease inhibitors to prevent the proteolytic breakdown of the antigen.
27. Gel overloading	Overloading the gel with too much protein can cause the development of "Ghost bands." Optimise protein concentration.
28. Insufficient blocking	Extend the blocking time.
29. Low antigen concentration	Consider immunoprecipitating target protein.
30. Unspecific secondary antibody binding	Use secondary antibody only control. If bands develop use a different secondary antibody.
31. Analyte aggregation	Increase DTT concentration.
32. Post translational modification	Protein sample has multiple modified forms e.g. acetylation, methylation and phosphorylation.
33. Protein degradation	Target protein of interest degraded.
34. Splice variants	Could lead to the visualisation of multiple bands.
35. High primary antibody concentration	Use a lower concentration of primary antibody.

HIGH BACKGROUND

Problems	Explanation
36. Unresolved proteins	Inefficient separation. Use high molecular weight and low molecular weight proteins.
37. Smile/Curve effect on the gel	Incorrect voltage. Inconsistent temperatures.

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