abcam

Ab131366 - VeriBlot for IP Detection Reagent (HRP)

Troubleshooting Guide

Problem	Possible Cause	Solution
Non-specific bands	Too much HRP in the system	Dilute the VeriBlot for IP Detection Reagent (HRP)
	Too much primary antibody	Dilute primary antibody
	SDS caused nonspecific binding to protein bands	Do not use SDS during immunoassay procedure
Speckled background on film	Aggregate formation in the VeriBlot for IP Detection Reagent (HRP)	Filter detection reagent through a 0.2 µm filter or centrifuge and use supernatant
Weak or no signal	Too much or not enough HRP in the system	Optimize the VeriBlot for IP Detection Reagent (HRP)
	Insufficient quantities of antigen or antibody	Increase concentration of antibody or antigen
	Inefficient protein transfer	Optimize transfer
High Background	Used too much detection reagent	Dilute the VeriBlot for IP Detection Reagent (HRP)
	Inadequate blocking	Optimize blocking conditions
	Inappropriate blocking reagent	Try a different blocking reagent
	Inadequate washing	Increase length, number or volume of washes
	Film was overexposed	Decrease exposure time or use background eliminator
	Antigen or antibody is too concentrated	Dilute the antigen or antibody
Detection of denatured and blotted IgG	All IgG in the sample was not denatured	Increase reducing reagent concentration
		Boil sample to aid in reduction of IgG disulfide bonds
		Use denaturing electrophoresis conditions
Antigen of interest was not detected	Sample does not contain antigen or does not contain a detectable quantity of antigen	Optimize expression and lysis procedures
	Antibody does not recognize antigen	Use a different primary antibody
	Primary antibody is not compatible	Verify target by performing a dot blot using a traditional secondary antibody

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

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