Troubleshooting Guide for VECTASTAIN® ABC-AmP™ Kits

Problem	Possible Cause	Recommendation
Weak or no signal	Insufficient sample loaded on the gel or poor transfer efficiency	Load more sample, and optimize the electrophoresis and transfer protocols, use biotinylated protein molecular weight standards to test transfer efficiency
	Low affinity primary antibody, or too low concentration of primary antibody	Use positive control, prolong incubation in primary antibody, or use higher primary antibody concentration
	Incorrect biotinylated secondary antibody used	Use correct species-specific biotinylated secondary antibody
	ABC-AmP™ reagent blocked	Use diluent such as 1x casein that does not contain biotin
	Substrate not developing properly	Check enzyme substrate in dot blot test
	Insufficient film exposure time when using DuoLuX™ as substrate	Optimize the exposure time
Excessive or diffuse signal or "reversed bands"	Too much protein loaded on the gel	Load less sample, and optimize the electrophoresis step
	Too high concentration of primary antibody	Optimize the primary antibody concentration
Non-specific bands	If bands evenly distributed in all lanes, sample buffer possibly contaminated with keratin	Use freshly prepared sample buffer
	Endogenous biotin-containing protein in the sample, or presence of non-specific streptavidin-binding protein elements	Use Streptavidin/Biotin Blocking kit (Cat. No. SP-2002) before primary antibody incubation
	Sample contains immunoglobulin protein elements that are being detected by the secondary antibody	If using a mouse primary antibody to probe protein sample of mouse origin, use Vector Mouse on Mouse Basic Kit (Cat. No. BMK-2202)
	Areas of the membrane dried during the detection	Always keep the entire membrane immersed in solution

Problem	Possible Cause	Recommendation
BCIP/NBT substrate develops in staining dish solution	ABC-AmP™ reagent still present in staining dish	Use separate staining dish for ABC-AmP™ reagent and substrate steps
Uneven background	Membrane dried during detection	Always keep the entire membrane immersed in solution
High but even background	Film exposure too long when using DuoLuX™ as substrate	Optimize the exposure time
	Insufficient blocking or washing	Prolong blocking step Dilute primary antibody in 1x casein solution Use 1x casein solution in the blocking and wash steps, and as diluent for detection reagent Use fluorescence visualization of DuoLuX™ substrate

7/03

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