

ProGlow™ Western Blot Ladder Troubleshooting

Observation	Cause	Solution
High background	Insufficient blocking	Prolong the blocking time; choose the suitable blocking reagents
	Contaminated membrane or transfer buffer	Wear gloves in the transfer process and keep the environment clean
	Concentration of primary antibody is too high	Increase dilution of primary antibody
	Temperature is too high during primary antibody incubation	Incubate at 4°C overnight
	Long time exposure	Shorten the time of exposure
Weak or no signal	Low amount of loaded standards	Load a higher amount of standard on the gel
	Incomplete or over transfer	Optimize the transfer time and current
	Low quality antibody or short time incubation	Replace with new antibody or extend incubation time
	Ineffective ECL reagents	Make sure the detection reagents are freshly made
Too strong or multiple bands	Too much primary or secondary antibody	Optimize the concentration of antibody
	Overloading	Reduce amount of standard loaded on to the gel
	Nonspecific binding	Improve blocking efficiency and choose specific antibody

Note: We recommend using a Tris-Tricine SDS-PAGE system for high quality photos.