



## TROUBLESHOOTING GUIDE - IMMUNOPRECIPITATION

Problem	Possible Cause	Possible Solutions
<b>Non-Specific Background</b>	Insufficient washing	<ul style="list-style-type: none"> <li>• Use more stringent washes.</li> <li>• Alternate wash buffers from high to low salt.</li> <li>• Use a different detergent</li> <li>• Have one wash be with distilled water</li> <li>• Increase the number and time of washes</li> </ul>
	Still frozen lysates	<ul style="list-style-type: none"> <li>• Do not freeze before use</li> </ul>
	High antibody concentrations	<ul style="list-style-type: none"> <li>• Decrease concentration</li> </ul>
	Non-specific binding to agarose beads or antibodies	<ul style="list-style-type: none"> <li>• Pre-clear lysates</li> </ul>
	Non-specific binding to Proteins	<ul style="list-style-type: none"> <li>• Preload precipitated antibody, then block remaining sites with BSA, gelatin, acetone powders or 5% nonfat dry milk</li> </ul>
	Aggregated proteins in lysate	<ul style="list-style-type: none"> <li>• Prior to adding the antibody, thoroughly centrifuge at 100,000 x g for 30 minutes</li> </ul>
	Bridging Antibody	<ul style="list-style-type: none"> <li>• Test the bridging antibody alone by immunoprecipitation</li> </ul>
<b>Specific Background</b>	Polyclonal antiserum- protein complexes formed	<ul style="list-style-type: none"> <li>• Use a monoclonal or affinity purified polyclonal</li> </ul>
	Antigen consists of more than one polypeptide chain	<ul style="list-style-type: none"> <li>• The antigen may already consist of more than one polypeptide chain</li> </ul>
	Monoclonal or affinity purified polyclonal antibody recognizing homologous epitope	<ul style="list-style-type: none"> <li>• Use a monoclonal with a different epitope.</li> </ul>
	Immunoblots are actually Ig light or heavy chains	<ul style="list-style-type: none"> <li>• IgG light chains are recognized at ~28kDa, IgG heavy chains are recognized at ~55kDa</li> </ul>
<b>Specific Antigen Not Detected</b>	Non-suitable antibody	<ul style="list-style-type: none"> <li>• Try a different antibody. Sometimes polyclonals work better</li> </ul>
	Antibody concentration too low	<ul style="list-style-type: none"> <li>• Increase the concentration</li> </ul>
	Weak binding to the proteins	<ul style="list-style-type: none"> <li>• Use a bridging antibody to capture immunocomplex</li> </ul>
	Too many proteins in mixture	<ul style="list-style-type: none"> <li>• Centrifuge lysate at 100,000 x g for another 30 minutes to remove any extra fragments</li> </ul>
	Protein only available in low levels in the sample type	<ul style="list-style-type: none"> <li>• Increase antibody concentration</li> <li>• Increase cell lysate concentration</li> <li>• Metabolically label cellular proteins</li> </ul>

<b>Problem</b>	<b>Possible Cause</b>	<b>Possible Solutions</b>
<b>Specific Antigen Not Detected (Cont.)</b>	Other Interfering substances	<ul style="list-style-type: none"> <li>• Be cognizant of pH, excessive detergent concentrations, and reducing agents such as DTT, and <math>\beta</math>-mercaptoethanol</li> </ul>
	Incorrect bead type used	<ul style="list-style-type: none"> <li>• Make sure the right ones were used and re-try</li> </ul>
	Immune complex was stripped from agarose beads by wash buffer	<ul style="list-style-type: none"> <li>• Use a milder wash buffer</li> <li>• Change detergents to something with less salt and/or a lower detergent concentration</li> <li>• Reduce number of washes</li> </ul>
	Incubation times too short	<ul style="list-style-type: none"> <li>• Change the incubation time frame so that it can sit overnight at 4°C</li> </ul>
	Antigen destroyed or lost	<ul style="list-style-type: none"> <li>• Only use fresh lysates that you have prepared yourself</li> </ul>