

## Immunoprecipitation Troubleshooting Guide

PROBLEM	POSSIBLE CAUSE	SOLUTION
Non-specific background	Substances in sample bind non-specifically to agarose beads or antibodies in general	Pre-clear lysate. This step may be repeated to further reduce background.
	Non-specific binding to Protein A or G	Preload precipitated antibody, then block remaining sites with BSA, gelatin, acetone powders, or 5% nonfat dry milk. Store Protein A or G in 5% BSA.
	Aggregated proteins in lysate	Centrifuge lysate at 100,000g for 30 minutes prior to addition of antibody to remove aggregated proteins.
	Bridging antibody	Determine if bridging antibody is causing background by using it alone for the immunoprecipitation.
	Insufficient washing	Use more stringent washes. Try 1.0 M NaCl, 0.5 M LiCl, 1 M KSCN, 0.2% SDS, or 1% Tween 20. Alternating wash buffers from high to low salt or using different detergents may help. Try washing with distilled water for one wash.
		Increase the number of washes. Leave solid phase in the wash buffers for 10 minutes at each wash.
	Lysates frozen before use	Do not freeze lysates before use.
	Antibody concentration is too high	Decrease antibody concentration.
Specific Background	Antigen consists of more than one polypeptide chain	If specific proteins remain, remember that the antigen may consist of more than one polypeptide chain.
	Monoclonal or affinity purified polyclonal antibody recognizing homologous epitope	Try a monoclonal antibody with a different epitope.
	Polyclonal antiserum forming complexes with additional proteins	Try a monoclonal or affinity purified antibody.
	In immunoprecipitation-immunoblots bands are actually Ig light or heavy chains	IgG heavy chains appear at ~55kDa, light chains appear at ~28kDa.
Specific antigen not detected	Antibody not suitable for immunoprecipitation	Try a different antibody. Polyclonal antibodies may perform better than monoclonal antibodies.
	Incorrect bead type used	Verify correct bead type was used. See Table 2 and 3 on page 157.
	Antibody binds weakly to either Protein A or G	Use bridging antibody (e.g. goat anti-mouse IgG) to enhance immunocomplex capture.
	Protein of interest is found in low levels in the sample type	Increase precipitating antibody concentration.
		Increase cell lysate concentration. Metabolically label cellular proteins.
	Wash buffer stripped immune complex from agarose beads	Use milder wash buffer. Reduce salt and detergent concentration or use a different detergent. Reduce number of washes.

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Specific antigen not detected	Incubation times too short	Incubate with precipitating antibody for several hours at 4°C. The Protein A/G incubation should occur overnight.
	Antibody concentration too low	Increase precipitating antibody concentration.
	Too many competing proteins in the sample	Centrifuge lysate at 100,000g for 30 minutes before addition of antibody to remove insoluble proteins, membrane fragments, etc. and enrich the sample for the protein of interest.
	Antigen of interest lost or destroyed	Prepare fresh lysates. Avoid using frozen lysates. Add appropriate protease inhibitors to sample.
	Interfering substances in sample	DTT, β-mercaptoethanol or other reducing agents may destroy antibody function. Extremes in pH and excessive detergent concentrations may interfere with antibody-antigen interaction.