Immunoprecipitation TroubleShooting Guide

Problem	Possible Cause	Solution
No binding	Antibody not capable of immunoprecipitation	Try a different antibody. Polyclonal antibodies generally perform better than monoclonal antibodies.
	Insufficient amount of primary antibody used	Determine optimal concentration of primary antibody by titration.
	Too many competing proteins in sample	Spin the lysate at 100,000g for 30 minutes before adding the antibody to remove insoluble proteins, membrane fragments, etc.
	Antigen of interest not present	Make sure sample is appropriate.
	Antigen of interest lost or destroyed in sample	Prepare fresh lysates. Avoid using frozen lysates. Use appropriate protease inhibitors in sample.
	Washes too stringent	Reduce the number of washes. Reduce salt and detergent concentration or use a different detergent.
	Incubation times inadequate	Incubation times should be appropriate for the system. Generally, the primary antibody and antigen of interest are incubated 1 hour to overnight at 2-8°C
	Protein A, G or L used may not bind species or subclass of primary antibody	See Protein A/G/L selection guide.
	Interfering substances present in sample	Lysates containing dithiothreitol, 2-mercaptoethanol or other reducing agents will destroy antibody function and must be avoided. Extremes in pH and excessive detergent concentrations can also interfere with the antibody-antigen interaction.
High background or unwanted proteins precipitate	Substances in sample bind non-specifically to either agarose beads or antibodies	Include a pre-clear step by incubating the lysate with the Protein A/G/L agarose conjugate without the antibody. Or, if using, for example, product A1205, mouse monoclonal anti-FLAG M2 resin, the samples would be pre-incubated with mouse IgG-agarose (A0919). This will help to remove anything that might otherwise non-specifically bind to either agarose or mouse IgG.
	Non-specific binding to Protein A, G or L agarose beads	Add saturating amounts of competitor proteins. For example, add 2% BSA to protein-A agarose beads.
	Concentration of antibodies too high	Determine optimal concentration of antibody by titration.
	Inadequate washing	Use more stringent washes. Try 1.0 M NaCl, 0.5 M LiCl, 1 M KSCN, 0.2% SDS or 1% Tween 20. Consider using distilled water wash as one wash. Increase the number of washes. Transfer the pellet to a fresh tube prior to the last wash.