Troubleshooting Guide Immunoprecipitation and Co-IP

Problem	Reason	Suggestions
High background / Unspecific binding	Lysis step	
	Overheating of sample during sonication	Keep samples on ice, reduce pulse duration, and extend periods between pulses. Avoid overheating of sample during sonication. This may lead to thermal damage or fragmentation of protein of interest or may harm the epitope.
	Protein aggregation	Use fresh cells if possible; avoid frozen cells. If frozen material has to be used, use frozen lysate (flash freeze in liquid nitrogen and store at -80°C). Remove protein aggregates by centrifugation at full speed for 30 minutes.
	Protein degradation and/or proteolysis during cell lysis	Keep samples on ice and add protease inhibitors. When working with phosphoproteins also add phosphatase inhibitors.
	Whole cell lysate too complex	Reduce complexity of lysate by extracting protein of interest from a certain cellular compartment (e.g. nuclei).
	Binding step	
	Buffer incompatibility	Please refer to Nano-Trap manual for a list of compatible buffer components. If a specific compound is not listed, optimize its concentration.
	Detergent concentration too high	Reduce detergent concentration in incubation buffer: recommended final concentration is 0.1 % (e.g. NP-40 or Triton X-100). Please also refer to Nano-Trap manual for a list of compatible detergent concentrations.
	Non-specific binding of proteins to beads (matrix)	Beads are not pre-blocked. Pre-block beads with 1-3 % BSA for 1-2 h at +4°C. Wash beads 3-4 times with wash/dilution buffer before use. Reconstitute beads for long-term storage again 1:1 in 20 % Ethanol.
		Pre-clear lysate using ChromoTek binding control beads.
		Shorten incubation step to 60 minutes.
		Try different matrix.
	Too many cells/too much protein in lysate leading to a lot of non-specific proteins in eluate	Reduce number of cells/lysate used. It is recommended to use max. 500 µg cell lysate per IP reaction.
	Washing step	
	Wash steps not sufficient	Prolong washing steps (5-10 minutes) and perform more than 3 wash steps (5-10 times).
		Increase buffer volume during washing steps.
	Poor mixing of the beads during washing procedure	Wash beads by pipetting up and down or by inverting the tube several times.
	Wash buffer not stringent enough	Test various salt concentrations (150 mM - 500 mM) in wash/dilution buffer to remove unspecific hydrophilic proteins.
		Add a non-ionic detergent (Tween 20 or Triton X-100) to the wash/dilution buffer, in concentrations between 0.01–0.1%.
	Insufficient wash buffer removal after immunoprecipitation	Remaining wash buffer can contain unwanted proteins and gives rise to unwanted background.
	Unspecific binding of proteins to the tube	Transfer beads to new tube during last washing step to avoid carry-over contamination. Use siliconized or “low binding” tubes.
Weak / no pull down of protein of interest	Protein of interest is not recognized by the Nano-Trap	Check specificity table at www.chromotek.com or contact info@chromotek.com.
	Low or no expression of protein of interest	The higher the target protein concentration, the higher the IP yield. Check expression profile of the target protein by Western Blot. If target protein is expressed at low levels, increase amount of lysate used. NB: this may increase non-specific binding.
	Proteolysis and denaturation of proteins	Add protease inhibitors during lysis and immunoprecipitation and keep samples on ice or at 4°C at all times.
	Protein of interest is insoluble	Check whether protein is soluble in lysis buffer. Control input fraction before and after centrifugation by Western blot.
	Interfering substances during binding step	Lysates containing dithiothreitol (DTT), 2-mercaptoethanol or other reducing agents may harm single-domain antibody (V_HH, nanobody) and should be avoided.
	Incubation time too short	Prolong incubation time of Nano-Trap with lysate.
	Poor or no cell lysis	Choose lysis buffer suitable for your cells of organism. Control lysis by SDS-PAGE/Coomassie blue staining.
	Beads settle during IP	Make sure beads do not settle during incubation with lysate. Bead settling during incubation will result in inefficient IP.
	Not enough beads used per IP reaction	Make sure that Nano-Trap beads are resuspended well by carefully pipetting up and down a few times. Do not vortex the beads, as this could damage the Nano-Trap. Cut off the top of the tip when pipetting agarose beads.
	Beads removed during equilibration or wash steps	Check whether beads have settled completely before you carefully remove supernatant.
	Loss of beads because they do not settle during centrifugation	Add detergent to buffer to reduce surface tension. Change tube and increase centrifugation speed up to 5,000 g for max 5 minutes. Use spin columns to reduce loss of beads.
	Beads adhere to the tube wall	Use buffer with detergent.

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Problem	Reason	Suggestions
	Inaccessible epitope	Reduce steric hindrance of interaction between Nano-Trap and protein of interest by using long and flexible linker sequences for your fusion protein construct.
	Wash conditions are too stringent	Decrease detergent and/or salt concentration in wash/dilution buffer.
	Wash steps too long	Shorten wash time.
	Incubation volume too large during IP	Reduce incubation volume during IP.
Poor or no elution of protein with glycine (low pH)	Elution efficiency depends on actual protein and may not be sufficient	Ensure that glycine buffer is at correct concentration and pH. Increase elution efficiency by constantly pipetting up and down for 30-60 seconds. Repeat elution step. Do not forget to adjust pH immediately after elution! Boil beads following elution in reducing 2x SDS-sample buffer to confirm efficiency of elution. Analyze supernatant by SDS-PAGE and Western blot to confirm presence of protein.
Co-IP not successful	Interacting protein is not present	Conduct Western blot to control whether interaction partner is expressed.
	Lysis and binding step	
	Protein-protein interaction has been disrupted during freezing of cells	Use fresh cells if possible; avoid frozen cells.
	Buffer components and/or concentrations are too stringent and disrupt or inhibit protein-protein interaction	Cell lysis is a critical step in Co-IPs, make sure to use a suitable lysis buffer. RIPA buffer can denature your protein of interest and may disrupt the protein-protein interaction. For Co-IP of soluble proteins, use a non-detergent, low-salt lysis buffer. This mild lysis buffer is probably least likely to interfere with protein-protein interactions. For less soluble protein complexes, add non-ionic detergents such as NP-40 or Triton X-100 to lysis buffer. Try different binding conditions by testing various detergent and salt concentrations suitable for the protein-protein interaction.
	Additives or a ligand needed for protein-protein interaction missing	If required, add additives or a ligand to binding buffer in order to facilitate protein-protein interaction.
	Incubation time too long	Shorten incubation time, some interactions/protein complexes are only transient or unstable. If you want to detect low-affinity or transient interactions, you may add a cross-linking step.
	Washing step	
	Buffer components and/or concentrations are too stringent and disrupt or inhibit protein-protein interaction	Make sure you are using an appropriate wash buffer. Use less stringent wash buffer conditions and reduce number of wash steps. Detergents, salts and other additives may reduce non-specific binding but may also decrease yield. The washing step often needs to be improved to determine the level of stringency that does not disrupt the protein complex. Each protein complex requires its own wash buffer composition for successful Co-IP, and it is not possible to predict buffer composition required for isolation of protein complex. Save the used wash buffer from each washing step to track if the protein of interest and its interacting partners were depleted by washing.
Inefficient IP after PFA cross-linking	Fixation time too long and epitope is not accessible anymore	Make sure that your IP-reagent still recognizes your protein of interest after cross-linking. Carry out a fixation time course for your cell line to determine the optimal fixation time for it and the epitope of interest. Cell lines and epitopes differ in both fixation efficiency and sensitivity to fixation reagents.
	Overheating of sample	Keep sample on ice, reduce pulse duration, and extend periods between pulses.
	Paraformaldehyde interferes with protein-protein interaction	Use methanol-free formaldehyde to avoid over-fixation.
	Temperature too high during cross-linking	Watch cross-linking temperature. Fixation is diffusion dependent and therefore affected by temperature.

Additional Support

Please also refer to FAQ section and fluorescent protein specification table at www.chromotek.com or contact support@chromotek.com.