**Troubleshooting**

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| **Step** | **Problem** | **Possible reason** | **Solution** |
| 1.1-1.8 | No antibody or only small portion is coupled to beads. | pH of sodium borate solution is incorrect. | Make sure all sodium borate is dissolved before use, and pH is adjusted to the correct value. |
|  |  | DMP is degraded. | Light and moisture rapidly inactivate DMP. Buy and use small amounts that are stored dry (with desiccant). Allow DMP to come to room temperature before opening to prevent condensation. |
|  |  | Incorrect sepharose beads | Check the binding strength of the antibody to the particular type of sepharose, such as protein A or protein G coupled sepharose. |
| 2.9 | Recovery of bait is low. | Antibody does not bind bait specifically. | Make sure that the antibody recognizes the bait protein specifically by including positive and negative controls, e.g. overexpress the bait protein in a cell system and if available compare signal with a knockout strain or a cell or tissue that does not express the bait protein. |
| 2.9 |  | Antibody does not recognize native epitope. | Even though an antibody may work well for western blotting, it may not recognize the epitope in non-denatured protein. Try a different antibody for the bait. |
| 2.1-2.23 |  | Too little antibody coupled beads were used or too many beads were lost during procedure. | Use an insulin syringe to remove solutions during washing of the Co-IP. Check that sufficient quantities of antibody have been coupled to the beads and enough beads were used for the Co-IP procedure. |
| **2.1-2.23** |  | pH of solutions was incorrect. | All solutions need to have the correct pH, in particular the lysis buffer and elution buffer. It is critical that detergent is added to the elution buffer for elution of membrane proteins. |
| **2.1** |  | Not enough starting material. | Increase the starting material amount. “The more the merrier” still holds true for Co-IP experiments if low abundant proteins are used as bait.  Confirm presence of the protein in the starting material. |
| 3 |  | liquid chromatography or mass spectrometer problem | Ensure that chromatographic separation is good and the mass spectrometer is calibrated correctly. Run a test sample if necessary. |
| 3 |  | Wrong protein database used for search | Download the correct protein database for the species that was used in the experiment, it should contain the bait protein of interest. |
| 2.9 | Many background proteins were identified, but little of the bait. | Antibody is not suitable for IP. | If the antibody binds to many other proteins in addition to the bait, it may not be suitable for Co-IP. Try a different antibody if available or affinity purification of an antiserum. |
| 2.10- 2.12 |  | Insufficient washing of the IP. | Carry out all five wash steps and remove washing solution carefully. If necessary an additional wash step with lysis buffer containing no detergent can be included. Washing helps to increase signal to noise ratio. |
| 2.7 |  | Pre-clearing was not sufficient. | Pre-clear the cell lysate before incubation with antibody-coupled beads. Remove insoluble material by centrifugation and be careful to remove it completely. |
| 4 | CoPIT cannot distinguish background from specific interactors. | Too much background was present in the IPs or bait was not enriched enough. | Optimize experimental procedure. |