

Detailed troubleshooting for immunoprecipitation.

High background

Carry over of proteins that are not detergent soluble

Remove supernatant immediately after centrifugations. This should leave insoluble proteins in the pellet. If resuspension occurs, centrifuge again.

Incomplete washing

Wash well at relevant stages by placing a lid on the tube and inverting several times before centrifuging.

Non-specific proteins are binding to the beads

Beads are not pre-blocked enough with BSA. Make sure BSA (fraction V) is fresh and incubate fresh beads for 1 hr with 1% BSA in PBS. Wash 3-4 times in PBS before using them.

Antibody used contains antibodies that are not specific enough

Use an affinity purified antibody, preferably pre-adsorbed.

Too much antibody used leading to non-specific binding

Check the recommended amount of antibody suggested. Try using less antibody.

Too many cells/too much protein in lysate leading to a lot of non-specific proteins in eluate

Reduce the number of cells/lysate used. We recommend using 10-500 µg cell lysate.

Non-specific binding of proteins to antibody

If there are many proteins binding non-specifically, then try reducing the amount of sample loaded onto the beads. You can also pre-clear the lysate by pre-incubating the prepared lysate with the beads before commencing with the immunoprecipitation (please see the protocol). This should clear the lysate of any proteins that are binding non-specifically to the beads. Some researchers also use an irrelevant antibody of the same species of origin and same Ig subclass to pre-clear the lysate.

Antigen degrading during immunoprecipitation

Ensure fresh protease inhibitors are added when sample is lysed.

High amount of antibody eluting

Too much antibody eluting with the target protein

Try reducing the amount of antibody. Crosslinking the antibody to the beads before the immunoprecipitation and eluting using a gentle glycine buffer gradient should significantly reduce the amount of antibody eluted.

No eluted target protein detected

Target protein not expressed in sample used/low level of target protein expression in sample used

Check the expression profile of the target protein to ensure it will be expressed in the cells of your samples. If there is low level of target protein expression, increase the amount of lysate used. However, this may result in increased non-specific binding so it would be advisable to pre-clear the lysate before commencing with the IP procedure.

Insufficient antibody for capture of the target protein

Check that the recommended amount of antibody is being used. The concentration of antibody may require increasing for optimization of results.

Target protein has not eluted from the beads

Ensure you are using the correct elution buffer and that it is at the correct strength and pH for elution of the protein.

Antibody has not bound to immunoabsorbant beads

Ensure you are using the correct beads for the antibody isotype used.

Incorrect lysis buffer used

Check datasheet to see if the antibody detects denatured or native protein and ensure the correct lysis buffer is used.

Protein of interest is obstructed by antibody heavy or light-chains

Secondary antibody recognizes denatured/reduced primary antibody released during the IP procedure or endogenous IgGs

Using secondary antibodies, recognizing the heavy and light-chain of the primary antibody for WB detection of IP samples will always result in two bands (the heavy-chain at 50kDa and the light-chain at 25kDa).

To avoid interference by the antibody chains we recommend using secondary antibodies, such as VeriBlot for IP, that specifically recognize native (non-reduced) primary antibodies.