High Background

Possible Causes	What can you do?
Inadequate washing	Use a more stringent washing buffer. Try to use a high salt washing buffer or add 0.2% SDS or 1% Tween20 to washing buffer. Increase the number of washes.
Concentration of antibodies too high	Check the recommended amount of antibody as indicated in the datasheet. Titrate and optimize the optimal antibody amount used per IP experiment.
Non specific binding to Protein A,G or L	Pre-block beads with BSA. Incubate beads with 2%BSA in PBS for 1 hour wash in PBS before use.
Non specific binding to agarose beads	Include a pre-clear step by incubating lysate with Protein A/G/L agarose beads.
Antibody not specific enough	Use affinity purified and pre-absored antibody for IP experiments.
Sample degradation	Add adequate protease inhibitors and phosphatase inhibitors throughout sample preparation and Ip steps.

Interference from heavy or light chain

Possible Causes	What can you do?
Secondary antibody rcognizes heavy / light chain denatured from primary antibody	Use secondary antibodies which only recognizes native form of IgG for immunoblotting.

No binding

Possible Causes	What can you do?
Insufficient antibody	Check the recommended amount of antibody as indicated in the datasheet. Titrate and optimize the optimal antibody amount used per IP experiment.
Washes too stringent	Reduce the number of washes. Reduce salt concentration in the wash buffer.
Incorrect Protein A/G/L used	Make sure that the Protein A/G/L beads are capable of binding to the antibody subclass being used.
Target protein not present in the sample used	Make sure that the target protein is expressed at a relatively high level in the sample used by including an Input sample in the WB.
Incorrect Lysis buffer used	Make sure that the lysis buffer used is not over-denaturing and destroy the native conformation of the target proteins.
Antibody not capable of immunoprecipitation	Try a different antibody. Try polyclonal antibody if monoclonal antibody does not work well.