

Troubleshooting and FAQ's for Immunoprecipitation with Dynabeads[®]

- [Immunoprecipitation with Dynabeads[®] does not work.](#)
- [I was able to immunoprecipitate my protein using Dynabeads[®] before, but after cross-linking the antibody to the beads, I get no protein bands on my gel.](#)
- [I have cross-linked my antibody to the Dynabeads[®], but there are still antibodies coming off the beads during elution.](#)
- [I removed the non-cross-linked antibodies before performing the immunoprecipitation step, but still get bands on gel indicating that the antibody is coming off the Dynabeads[®].](#)
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Immunoprecipitation with Dynabeads[®] does not work.

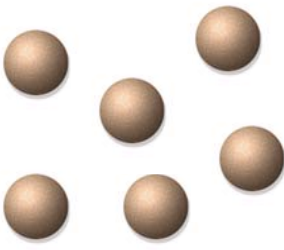
- Verify binding/specificity of your antibody to your antigen, e.g., by ELISA.
- Check the binding of your antibodies to the beads. If the antibodies are not captured and bound to the beads, the immunoprecipitation experiment will not work.
- If you have used the indirect method, try the direct method. Conversely, if you have used the direct method, try the indirect method.
- Check the amount of beads and sample volume. With reference to the capacity of different beads proposed in the package inserts, increase the amount of beads or the concentration of your antibody during coupling.
- Increase the incubation time.
- Try another antibody.

I was able to immunoprecipitate my protein using Dynabeads[®] before, but after cross-linking the antibody to the beads I get no protein bands on my gel. The binding sites of your antibody might be affected by the cross-linker and your antibody does not recognize the antigen anymore.

- Try IP without cross-linking the antibody to the beads.
- Try a different cross-linker.
- To prevent co-elution of antibody, try one of our surface-activated Dynabeads[®]. This allows you to conjugate the antibody to the beads directly, through covalent binding.

I have cross-linked my antibody to the Dynabeads[®], but there are still antibodies coming off the beads during elution. Cross-linking will never be 100%. Some antibodies are not cross-linked and may come off under elution.

- Perform a washing step with low pH directly after cross-linking to remove non-cross-linked antibodies. Remember to bring the pH back to normal before IP.



I removed the non-cross-linked antibodies before performing the immunoprecipitation step, but still get bands on gel indicating that the antibody is coming off the Dynabeads®.

- If you are using reducing agents in the sample buffer before gel loading, try incubating the beads in a sample buffer without reducing agents. Reducing agents such as DTT or β -mercaptoethanol will reduce disulfide bridges and result in release of antibody light and heavy chains.
- You may also elute the protein by lowering the pH, to leave the antibody bound to the beads.

I experience non-specific binding in my immunoprecipitation experiment.

- Use more stringent washing buffer for washing.
- Add a non-ionic detergent (Tween-20 or Triton X-100) to the washing buffer, in concentrations between 0.01-0.1.
- If the beads are blocked before precipitation, add identical blocker to the washing buffer.
- Increase the number of washing steps.
- Prolong the washing steps.
- Decrease incubation time (beads and sample).
- Try the indirect method.
- Decrease the antibody concentration.
- A pre-clearing step may be performed to remove molecules that non-specifically bind to the protein A/protein G or the beads themselves.

Are Dynabeads® Protein A and Protein G pre-blocked with BSA?

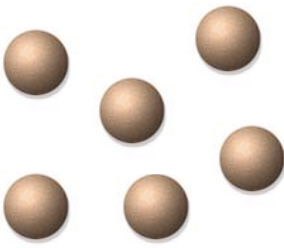
No. The Dynabeads® are coupled with protein A or protein G. The hydrophilic surface is not blocked with BSA.

How can I block Dynabeads® Protein A and Protein G with BSA?

Blocking with BSA will work best on a hydrophobic surface, where surface adsorption keeps the blocker in position. Dynabeads® Protein A and Protein G have a hydrophilic surface. Hence, BSA blocking may not be very successful, as surface adsorption is not promoted between the hydrophilic surface and the hydrophobic BSA. Instead, reduce non-specific binding by performing more stringent washing and adding Tween-20 detergent (concentrations of 0.01-0.1%) to the washing buffer.

I have used biotinylated antibodies coupled to Dynabeads® Streptavidin for immunoprecipitation. How do I elute my target protein from the antibody without eluting the antibody off the beads?

- Use mild elution conditions, e.g., a buffer with high salt or low pH. Heating the beads at 95°C for 5 minutes in SDS buffer will elute the antibody as well.



Can I use the secondary coated Dynabeads® for immunoprecipitation?

Yes, we supply two secondary coated 2.8 micron beads for immunoprecipitation:

- Dynabeads® Sheep anti-Mouse (112.01/02) to be used when your primary antibodies are derived from mouse
- Dynabeads® Sheep anti-Rabbit (112.03/04) to be used when your primary antibodies are derived from rabbit serum

Can I use bigger (4.5 micron) Dynabeads® for immunoprecipitation?

Yes, you can use the bigger beads. You can use Dynabeads® Sheep anti-Rat (110.35) when your primary antibodies are of rat origin. The smaller beads do, however, provide larger surface area and will hence give higher yields of protein.

As negative control, I have incubated my sample with Dynabeads® Protein G that are not coated with antibody, but I get non-specific binding to the beads:

Using Dynabeads® Protein G (or Protein A) alone with your IP sample is not a good control. Different molecules in your sample will bind either to protein G or to the beads themselves through a variety of interactions (hydrophobic interactions, charge interactions etc). As a negative control, you may use Dynabeads® Protein G bound to an irrelevant IgG.

This info is to be found at:

<http://www.invitrogen.com/immunoprecipitation>

<http://www.invitrogen.com/DynabeadsProteinAG>