

Immunoprecipitation with Dynabeads® Protein A or Protein G

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Introduction

Immunoprecipitation (IP) is the technique by which a target protein or antigen is precipitated from a solution using an antibody (Ab) specific for that target. In addition to isolating a specific target protein from a sample, IP can also be used to isolate protein complexes from cell extracts by targeting a protein or antigen believed to be part of the complex.

Immunoprecipitation is used to:

- Estimate the molecular weight, identity, or quantity of a protein of interest
- Study protein-protein interactions
- Determine the concentration of proteins
- Analyze the expression level of a protein of interest

Monosized, superparamagnetic Dynabeads® (Figure 1A) with Protein A or Protein G coupled to their surface provide a suspendable solid support for immunoprecipitation of pure target proteins. The captured immune complexes are easily removed from the supernatant by magnetic separation (Figure 2). Dynabeads® magnetic separation technology allows for simple and efficient washing. The pure precipitate can then be eluted from the beads and analyzed by western blotting or mass spectrometry.

The procedure can be divided into the following stages:

1. Sample preparation
2. Immunoprecipitation
3. Elution

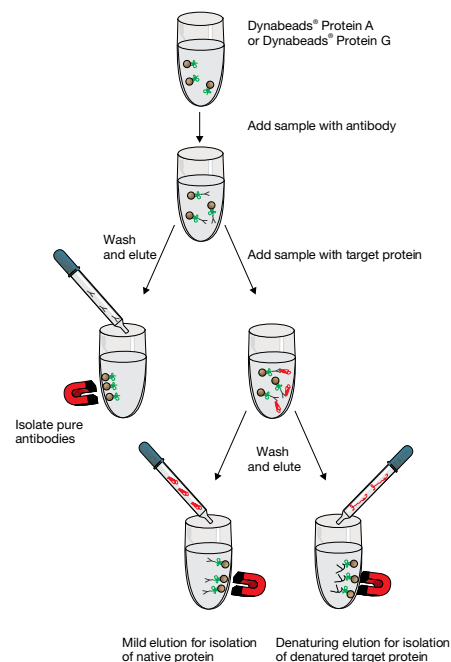


Figure 2—An antibody specific for the target protein/antigen is added to Dynabeads® Protein A or G. Captured antibodies are separated from the sample and may be eluted in a small volume and the beads reused. Dynabeads® with captured antibody facilitate small-scale IP and co-IP within minutes. Target proteins/antigens are eluted in a small volume. Depending on your elution protocol, the beads can be reused.

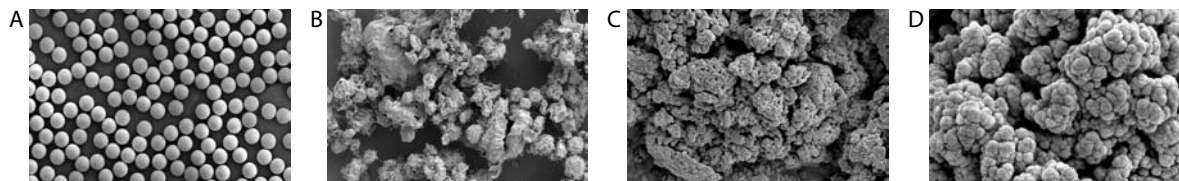


Figure 1—Monosized, superparamagnetic Dynabeads®. A. Manufactured with highly controllable product qualities, Dynabeads® are highly uniform within and between batches. **B–D.** Magnetic particles from alternative suppliers.

1. Sample preparation

Depending on your specific IP application and the location of the target antigen within the cell (nucleus, cytosol, membrane, etc.), develop an appropriate lysis strategy to maximize structural integrity. The ionic strength (salt concentration), choice of detergent, and pH of the lysis buffer may significantly affect the IP efficiency as well as the integrity of the target antigen.

The choice of detergent is crucial and may be influenced by many factors, e.g., the antigen's subcellular location and whether you would like to preserve subunit associations and other protein-protein interactions.

Proteolytic digestion can occur when lysosome contents are mixed with contents from other cell compartments. Regardless of the lysis strategy used, the lysis buffer should always contain protease inhibitors.

Following preparation of the lysate, determine the protein concentration and adjust to between 1 and 5 mg/ml with lysis buffer or phosphate-buffered saline (PBS). Two commonly used buffers for cell lysis:

- RIPA buffer gives lower background in IP. However, RIPA can denature some proteins. If you are conducting IP experiments to study protein-protein interactions, RIPA should not be used as it can disrupt the interactions.
- NP-40 buffer denatures proteins to a lesser extent, and is thus used for phosphorylation experiments when studying kinase activity. NP-40 is typically used for the study of protein-protein interactions. NP-40 is a nonionic detergent and the most commonly used detergent in cell lysis buffers for IP and westerns.

2. Immunoprecipitation

IP can be performed using a direct or indirect method.

Direct method

- The preferred method when the target protein is abundant
- Allows you to make up a stock of beads with bound Ab
- Requires less primary Ab

The primary Ab is first bound to the Dynabeads® Protein A or G as described in the product protocol. The beads and sample are then mixed and incubated at 2–8°C with tilting and rotation. Incubation time will depend upon the concentration of target protein and the specificity of the Ab toward this target (Table 1). When the concentration of target protein is high, a short incubation time (10 minutes) should be sufficient for target capture. Incubation can be prolonged, up to 1 hour, if necessary.

Indirect method

- The preferred method when the Ab has poor affinity for the target (Table 1) or when the target protein is in low abundance
- Requires minimal co-incubation of Dynabeads® with the sample, allowing for optimal control of background binding

The primary Ab is first incubated with the cell lysate to form an Ab-antigen complex. The complex is then captured by adding Dynabeads® Protein A or G to the sample, followed by magnetic separation. Care must be taken to avoid using an excess amount of Ab, as free Ab will bind to the beads much faster than will the Ab-antigen complex. Free Ab occupying binding sites on the beads may reduce protein yield.

Notes on buffer choice:

- Slightly alkaline pH and low ionic strength buffers typically favor protein solubilization.
- High salt concentration and low pH may cause the antigen to become denatured and precipitate out of solution.
- Non-ionic (e.g., Tween®, Triton® X-100, NP-40) or zwitterionic (e.g., CHAPS) detergents tend to preserve non-covalent protein-protein interactions.
- Ionic detergents (e.g., SDS and sodium deoxycholate) tend to have a denaturing effect on protein-protein interactions and may adversely affect the ability of the Ab to recognize the target antigen.

Note on optional preclearing:

Dynabeads® Protein A and Dynabeads® Protein G exhibit low nonspecific binding in most sample types. Certain samples may still require preclearing to lower the amount of nonspecific binders in the cell lysate, and to remove proteins with high affinity for Protein A or Protein G. In cases where preclearing is required, uncoated Dynabeads® Protein A or G are mixed with the sample under equivalent conditions. Preclearing should be kept to a minimum to retain the reproducibility and quantitative nature of the IP procedure.

To determine the optimal concentration, titrate the primary Ab. Based on the binding capacity of the beads, calculate the amount of beads required to capture the primary Ab. To ensure rapid kinetics and optimal protein capture, keep the sample concentrated during incubation. The volume of beads added should not be less than 1/10 of the sample volume.

Washing steps

The immunocomplexes captured on the Dynabeads® can be washed with RIPA, PBS, or similar buffers. The choice of buffer depends on your downstream application. RIPA buffer is more stringent compared to PBS, and when PBS is used a non-ionic detergent such as Tween® 20 (0.01–0.1%) should be added to reduce non-specific binding. Washing volumes should be adjusted depending on the volume of beads used in the IP, and to avoid loss of proteins and beads due to adsorption to the tube wall, we recommend repeated washes with smaller volumes (3 × 200 µl washes is suitable when starting with 50 µl of Dynabeads® Protein A or G).

Background binding

Background can arise from a variety of sources and can be caused by either specific or nonspecific binding. The following are suggestions on how to deal with nonspecific background:

- Use buffers with detergents to reduce hydrophobic interactions
- Use high salt to reduce ionic interactions
- Increase the number of washes
- Increase washing time
- Increase stringency of the washing buffer
- Decrease the incubation time (direct method)
- Apply the indirect method
- Decrease the concentration of primary Ab
- Include a preclearing step

Replace the primary Ab with an irrelevant immunoglobulin as a negative control. Note that Dynabeads® Protein A or G alone cannot be used as a negative control, as there will inevitably be some minor background binding to the Protein A or G, or to the bead surface.

Table 1—Binding characteristics of different immunoglobulins (Igs). Native Protein A and Protein G differ in their binding to Igs from different species and subclasses. For example, human IgG3 will bind strongly to Protein G, but only weakly to Protein A. S: strong binding; M: medium binding; W: weak binding; N: no binding.

Species	Ig class	Protein A	Protein G
Human	Total Ig	S	S
	IgG1, IgG2, IgG4	S	S
	IgG3	W	S
	IgD	N	N
	IgA, IgM	W	N
	Fab	W	W
	ScFv	W	N
Mouse	Total Ig	S	S
	IgG1	W	M
	IgG2a, IgG2b, IgG3	S	S
	IgM	N	N
Rat	Total Ig	W	M
	IgG1	W	M
	IgG2a	N	S
	IgG2b	N	W
	IgG2c	S	S
Goat	Total Ig	W	S
	IgG1	W	S
	IgG2	S	S
Sheep	Total Ig	W	S
	IgG1	W	S
	IgG2	S	S
Cow	Total Ig	W	S
	IgG1	W	S
	IgG2	S	S
Horse	Total Ig	W	S
	IgG(ab)	W	N
	IgG(c)	W	N
	IgG(T)	N	S
Rabbit	Total Ig	S	S
Dog	Total Ig	S	W
Cat	Total Ig	S	W
Pig	Total Ig	S	W
Guinea pig	Total Ig	S	W
Chicken	Total Ig	N	N

3. Elution

The Dynabeads®-Ab-antigen complex can be used further in co-IP experiments, or the antigen can be eluted for direct downstream analysis (e.g., SDS-PAGE followed by western blotting). The elution protocol should be chosen according to your specific downstream application (Figure 3).

Note that the affinity-bound primary Ab will co-elute with the target antigen; this will normally not interfere with downstream applications. To avoid co-elution of the Ab, it may be necessary to crosslink the primary Ab to Dynabeads® Protein A or G.

Milder elution methods can also be used to elute target protein from the beads. For example, protein can be eluted in low pH, such as in 0.1 M citrate (pH 2–3) for 2 minutes, to ensure full protein integrity when brought back to physiological pH.

Other Dynabeads® for IP

In addition to Dynabeads® Protein A and Dynabeads® Protein G, the following Dynabeads® can be used for IP:

- Streptavidin-Coupled Dynabeads®—for use with biotinylated Ab. Four different types of streptavidin-coupled Dynabeads® are available.
- Surface-Activated Dynabeads®—for covalent coupling of primary Ab directly to the beads. Preferred when co-elution of primary Ab with target protein is not desired. The Dynabeads® can be coated with Ab and stored for later use. Add 0.02% sodium azide (NaN₃) and keep refrigerated.
- Secondary-Coated Dynabeads®—for use with primary Ab. These beads have Ab specific for mouse or rabbit IgGs coupled to their surface, and may be used when the primary Ab is of mouse or rabbit origin.

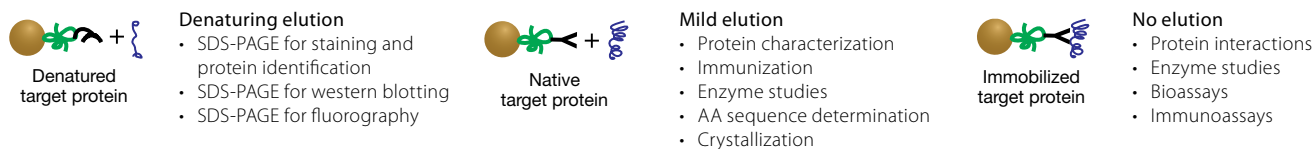


Figure 3—Choose an elution protocol according to your downstream application.

Notes on crosslinking:

- For crosslinking Ab, we recommend 5 mM BS₃ (bis(sulfosuccinimidyl) suberate (Pierce Cat. no. 21580) for optimal results, but other crosslinkers like DMP (dimethyl pimelimidate) or DSS (disuccinimidyl suberate) have also been used.
- Crosslinking is not 100% efficient. To prevent contamination by Abs that are not crosslinked, an elution step using low-pH buffer should be included. Remember to bring the pH of the bead suspension back to a normal level immediately after elution and before IP.
- Even after crosslinking, the use of reducing agents like DTT or β-mercaptoethanol in SDS-PAGE can reduce disulfide bridges of the Ab and cause the light chains of the Ab to be released during elution.
- Some of the binding sites within the Ab may get crosslinked. If loss of affinity after crosslinking is observed, avoid crosslinking. Alternatively, use one of our surface-activated Dynabeads® to allow covalent binding of the Ab to the beads.

Ordering information

Product	Concentration	Quantity	Cat. no.
Dynabeads® Protein A	~40 mg/ml	1 ml	100-01D
Magnetic beads coupled with recombinant protein A		5 ml	100-02D
Dynabeads® Protein G	~30 mg/ml	1 ml	100-03D
Magnetic beads coupled with recombinant protein G		5 ml	100-02D
DynaMag™-2		1 unit	123-21D
Magnet holding up to 16 standard 1.5–2 ml microcentrifuge tubes. Working volume: 10–2,000 µl			
DynaMag™-Spin		1 unit	123-20D
Magnet holding up to 6 standard 1.5 ml microcentrifuge tubes. Working volume: 10–1,500 µl			

To see our full range of Dynabeads® for immunoprecipitation, visit www.invitrogen.com/immunoprecipitation.

To find antibodies validated for immunoprecipitation, visit www.invitrogen.com/antibodies.