

Perform reproducible immunoprecipitation in less than 40 minutes

Dynabeads products



# Immunoprecipitation made easy with low nonspecific binding, high yield, and reproducibility

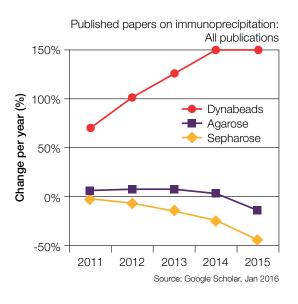


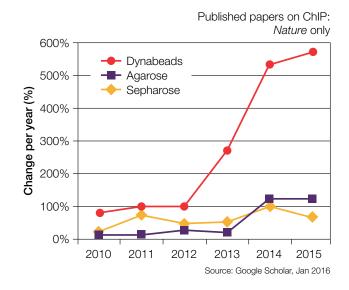
Magnetic beads have become the gold standard to use for immunoprecipitation (IP) and pull-downs because they are a faster, easier, and more efficient way of pulling down the proteins than traditional Sepharose™ agarose or other agarose resins.



IP is the small-scale affinity purification of antigens using a specific antibody, and one of the most widely used methods for antigen purification and detection. IP enables researchers to enrich for low-abundance proteins in order to improve downstream analyses, such as identifying the activation status, determining posttranslational modifications, or capturing protein-binding partners (co-immunoprecipitation, i.e., Co-IP). The target protein can also be bound to DNA (chromatin IP, i.e., ChIP) or to RNA (RNA IP, i.e., RIP) and be combined with sequencing or PCR assays.

We offer a wide variety of conjugated magnetic beads to meet most application and budget needs, including the most referenced technology—Invitrogen™ Dynabeads™ products.





#### **Highlights:**

- Low background—little to no nonspecific binding, and no preclearing
- Highly reproducible—uniform beads ensure the most consistent results
- **Highly sensitive**—Dynabeads technology is the most cited method for sensitive applications, such as ChIP and IP, of low abundance proteins
- Fast and easy—<40 min protocol with no centrifugation or preclearing steps
- Antibody savings—all binding occurs on the smooth outer surface of the beads which conserves precious antibodies and supports a cost-efficient solution per sample
- Flexible—products for IP, Co-IP, pull-down, and ChIP assays; ideal for both manual and automated protocols

#### Benchmarking: Dynabeads products vs. resin-based solutions

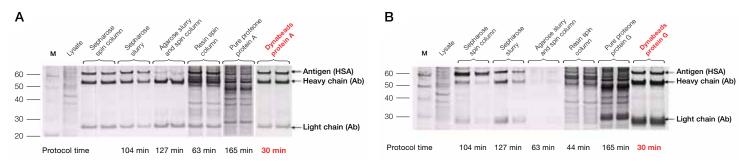


Figure 1. Shorter protocol time and better yields with Invitrogen Dynabeads. The same input of antibodies (Ab) and cell lysate was used for all IP protocols. With Dynabeads Protein A (left) and Dynabeads Protein G (right), all the antibodies on the bead surface are accessible for optimal, highly reproducible antigen binding.

Benchmarking: Dynabeads magnetic beads vs. other magnetic-based solutions

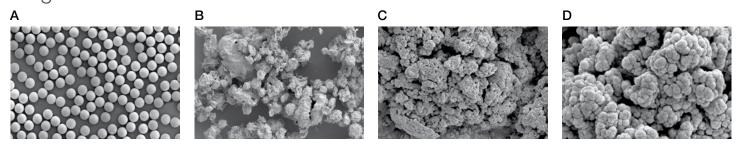
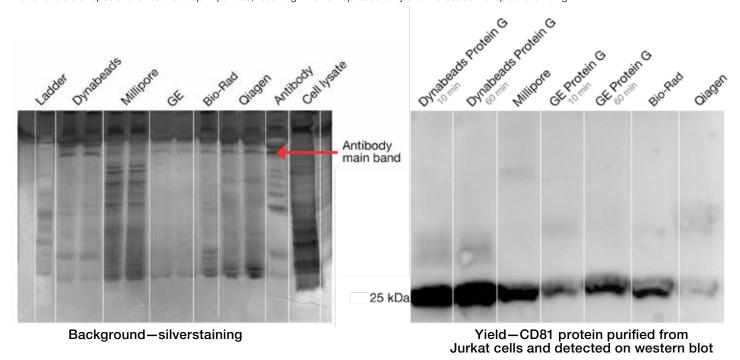


Figure 2. The magnetic bead you choose will affect your results. Dynabeads magnetic beads have a defined surface to carry out the necessary binding, with no inner surface to trap any unwanted proteins. (A) Dynabeads products are the most uniform, monodisperse superparamagnetic beads, manufactured with highly-controlled product qualities to ensure the highest degree of reproducibility. (B–D) Magnetic particles from alternative suppliers have variable shapes and sizes that trap impurities, resulting in lower reproducibility and increased nonspecific binding.



- Dynabeads Protein G provide very little nonspecific binding
- Dynabeads Protein G provide best signal/noise

- Dynabeads Protein G have the highest yield, even after only a 10-minute incubation with proteins
- Millipore also shows good yield, but with high nonspecific binding

Figure 3. Benchmarking data shows that Dynabeads magnetic products have the best performance when taking into account the combination of highest yield and lowest nonspecific binding. Our magnetic beads also have the fastest protocol, improving your lab life by removing unnecessary steps.

#### Choose your isolation strategy and find your product:

### Choose (1) if you have an antibody that recognizes your protein—

your choice of antibody binding products depends on whether your downstream assay method is mass spectrometry or if you don't want the antibody coeluted with your target protein.

Antibody binding is the most common method and is used when your target antibody can be bound directly to the beads or indirectly to a precoated ligand on the magnetic beads.

Choose (2) if you have a biotinylated antibody (or ligand) that recognizes your protein—main advantages for using a biotinylated antibody with streptavidin—coated beads for IP:

- If you have a sample rich in soluble IgGs
- If you have a recombinant antibody lacking Fc regions
- If you need a bead compatible with mass spectrometry (secondary– coated and epoxy–coated Dynabeads magnetic beads are also compatible with mass spectrometry)

### Choose (3) if you have a recombinant protein (fusion tag)—

the most popular fusion tags for recombinant protein expression are covered by Thermo Scientific™ Pierce™ and Dynabeads magnetic beads. These include His tag, GST tag, HA tag, and c-Myc tag. See thermofisher .com/iptags for product list.

Choose this if you have	Surface coating on the magnetic beads	Type of ligand required	Mass spec compatible	Non- specific binding	IP protocol time	Main benefits for IP	Products
	Protein A, G, or L	Primary antibodies from most species. Protein A, G, and L bind different antibody species and subclasses with different specificities.	No	Low	Dynabeads: <40 min Pierce beads: 130–180 min	Dynabeads — fastest, easiest protocol, low nonspecific binding	Dynabeads Protein A Dynabeads Protein G Immunoprecipitation Kit Dynabeads Protein A Immunoprecipitation Kit Dynabeads Protein G Pierce magnetic beads Protein A/G Pierce magnetic beads Protein L
(1) Antibody recognizes your protein	Secondary antibodies	Mouse IgGs or Rabbit IgGs	No**	Low	Dynabeads: ~40 min	<ul> <li>Fast and easy protocol</li> <li>Low nonspecific binding</li> <li>Specific binding of mouse or rabbit IgGs</li> </ul>	Dynabeads M-280 Sheep anti-Mouse IgG Dynabeads M-280 Sheep anti-Rabbit IgG
	Epoxy-coated beads*	Any protein ligand (e.g. antibody, peptide)	Yes	Ultralow	Dynabeads Ab coupling time: overnight; Co-IP protocol time: 30–40 min	Covalent coupling of the Ab gives ultralow nonspecific binding     No need for crosslinking     Gentle and efficient Co-IP of even large protein complexes	Dynabeads Antibody Coupling Kit Dynabeads Co- Immunoprecipitation Kit
(2) Biotinylated antibody	Streptavidin	Any biotinylated antibody or ligand	Yes	Low	30–40 min	Binds any biotinylated protein     For samples high in soluble IgGs     Recombinant Ab lacking the Fcregion	Dynabeads M-280 Streptavidin Dynabeads M-270 Streptavidin Dynabeads MyOne Streptavidin C1 Dynabeads MyOne Streptavidin T1
(3) Recombinant protein	Fusion tags	Different beads bind proteins with the following tags (His, GST, HA, c-myc)	Yes	Low	Dynabeads (Histag): ~25 min Pierce tags: ~70 min	<ul> <li>Purify many different proteins incorporated with the same tag</li> <li>No need for antibodies</li> </ul>	See thermofisher.com/ iptags for product overview

<sup>\*</sup>See more choices in surface-activated Dynabeads products for the binding and capture of additional targets.

<sup>\*\*</sup>Contains Tween-20 detergent that is contaminating for the mass spectrometry.

"Dynabeads are absolutely the best technology we have found so far for pulling out large complexes."

-Dr. Michael P. Rout, Rockefeller University

## Co-IP: With Dynabeads products you skip unnecessary steps and help ensure getting intact protein complexes

If you are using techniques such as Sepharose beads and spin columns for pull-down, note that your protein complexes can dissociate from exposure to large surfaces, mechanical strain (e.g., centrifugation), dilution, and excessive handling (preclearing). To preserve native protein conformations and large protein complexes, use the Invitrogen™ Dynabeads™ Co-Immunoprecipitation Kit. Just couple your antibody directly to the Dynabeads products, and use the magnet to separate your protein complexes.

Although some researchers choose to preclear using Sepharose beads, nonspecific binding can result in contamination.

### Advantages of Dynabeads products for protein complex isolation:

- Quick and easy pull-down of intact, functional protein complexes
- No time-consuming preparation steps
- Only isolate the proteins you want
- Can be adapted for high-throughput applications

#### 4 common IP myths debunked

Check out our myth-busting video series at: thermofisher.com/ipmyths

Myth	Fact
Background can't be avoided.	Almost all background is removed using Dynabeads magnetic beads because all antibodies are accessible on the smooth bead surface, limiting nonspecific background.
Preclearing is necessary to get good IP results.	Preclearing step is unnecessary with Dynabeads magnetic beads. You can save time and you use half the amount of solid phase, which helps save money.
Higher capacity is better for IP.	The high capacity of Sepharose beads comes from high surface area, which may also trap a lot of unwanted protein, thereby wasting antibody. Even with lots of washing, you will end up with unwanted background. Good capacity with high yield is best.
Dynabeads magnetic beads are expensive.	With no preclearing and less antibody used, Dynabeads magnetic beads help save you money by balancing optimal capacity/yield, reproducibility, and purity.

Product	Quantity*	Cat. No.
Dynabeads Protein A	1 mL	10001D
Dynabeads Protein G	1 mL	10003D
Immunoprecipitation Kit-Dynabeads Protein A	40 reactions	10006D
Immunoprecipitation Kit-Dynabeads Protein G	40 reactions	10007D
Pierce Protein A/G Magnetic Beads	1 mL	88802
Pierce Protein L Magnetic Beads	1 mL	88849
Dynabeads Antibody Coupling Kit	1 kit	14311D
Dynabeads Co-Immunoprecipitation Kit	40 reactions	14321D
Dynabeads His-Tag Isolation and Pulldown	2 mL	10103D
Dynabeads M-280 Sheep Anti-Mouse IgG	2 mL	11201D
Dynabeads M-280 Sheep Anti-Rabbit IgG	2 mL	11203D
Dynabeads M-280 Streptavidin	2 mL	60210
Dynabeads M-270 Streptavidin	2 mL	65305
Dynabeads MyOne Streptavidin C1	2 mL	65001
Dynabeads MyOne Streptavidin T1	2 mL	65602
*Most products are available in larger pack sizes		

#### **Resources:**

More information: thermofisher.com/immunoprecipitation

thermofisher.com/automation

FAQs: thermofisher.com/ipfaqs

Fusion tags: thermofisher.com/iptags

Videos: youtube.com/immunoprecipitation

Immunoprecipitation myth videos

Immunoprecipitation Publication Trends—The Reasons for the Shift

Immunoprecipitation Interactive Selection Guide

### Magnetic stands

Invitrogen<sup>™</sup> DynaMag<sup>™</sup> magnets isolate any target in combination with Dynabeads magnetic beads. Your waiting time is minimized as these powerful magnets quickly pull your Dynabeads-bound target to the tube wall. DynaMag magnets help ensure optimal working positions and are functionally adapted to suit your workflow.



### The most commonly used magnet for all molecular assays, including IP, is the DynaMag-2 magnet.

The Invitrogen™ DynaMag™-2 Magnet combines strong magnetic attraction with flexible ergonomic design

DynaMag-2 Magnet



Extra rack for DynaMag-2 Magnet

- Holds up to 16 standard 1.5 mL-2 mL microcentrifuge tubes in numbered spaces
- Rack can be removed and used to store tubes
- Rack makes it easy for resuspension, vortexing, rotation, or manual sample shaking: a center pin in the rack ensures equal vortexing of all tubes
- Efficient control and visibility of your proteins and nucleic acids isolation



#### Other magnets available for immunoprecipitation are:

#### Invitrogen<sup>™</sup> DynaMag<sup>™</sup>-Spin Magnet

Holds six 1.5 mL microcentrifuge tubes. Circular top rack can be quickly removed from the magnet in the base, ready for vortexing or manual sample shaking.

#### Plate magnets

Optimum working volume 5 µL-200 µL. See **thermofisher.com/magnets** for more information.

Product	Quantity	Cat. No.
DynaMag-2 Magnet	1 unit	12321D
SampleRack for DynaMag-2 Magnet	1 unit	12322D
DynaMag-Spin Magnet	1 unit	12320D
DynaMag-96 Bottom Magnet	1 unit	12332D
DynaMag-96 Side Magnet	1 unit	12331D
DynaMag-96 Side Skirted Magnet	1 unit	12027

#### Resources and ordering info for magnetic stands

More information: thermofisher.com/magnets

### invitrogen

Dynabeads
Y Antibody
Target protein
Nonspecific protein

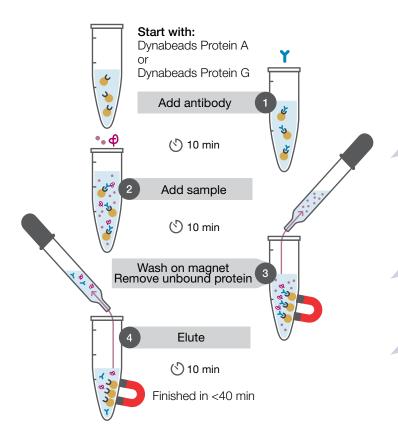


Figure 4. Immunoprecipitation in less than 40 min. Dynabeads magnetic beads precoupled with protein A or protein G act as a suspendable solid support that can be fixed by the use of a magnet. This allows for simple and efficient antibody capture, followed by immunoprecipitation of your pure target peptides, proteins, protein complexes, or other antigens.

"Dynabeads Protein G are so easy to work with and the background is minimal. I don't know what I would ever do without them now."

"I love how simple these are to use. They also save me a lot of time by not having to preclear or obsess about the wash steps."

"I love the ease of this reagent... faster and cleaner."

