Proteomics

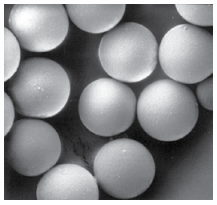
Perform reproducible immunoprecipitation in less than 40 minutes

Magnetic bead products

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

Immunoprecipitation (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 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.

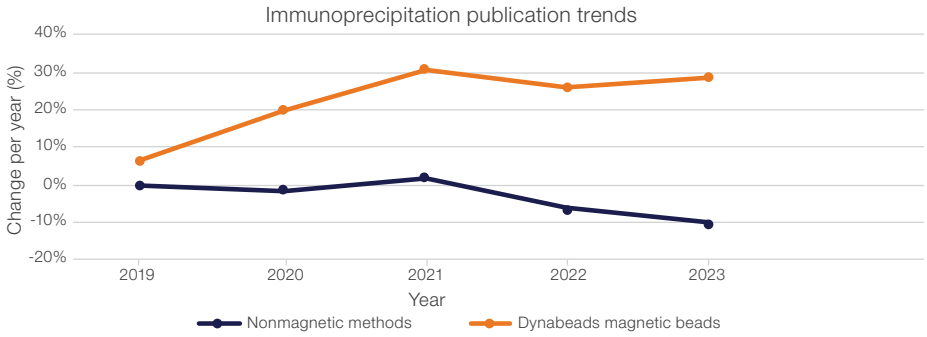
Magnetic beads have become an excellent choice to use for IP and pulldowns because they provide a faster, easier, and more efficient way of pulling down proteins than traditional Sepharose™ agarose or other agarose resins.

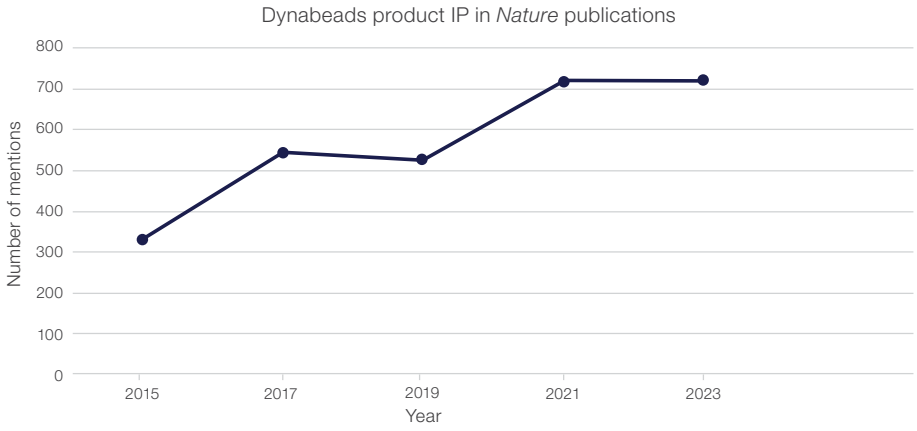


We offer a wide variety of conjugated magnetic beads to meet most application and budget needs, including the well known technology of Invitrogen™ Dynabeads™ products and the newly released Invitrogen™ DynaGreen™ products.

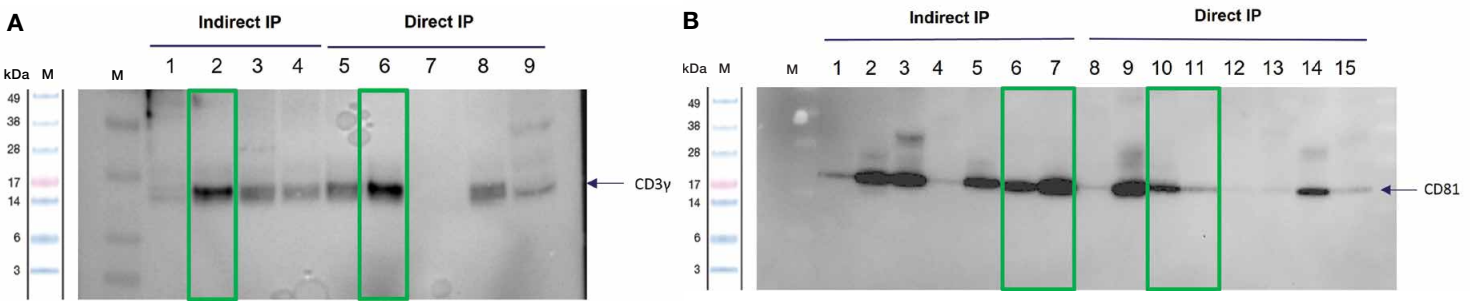
Dynabeads product highlights:

- **High purity**—little to no nonspecific binding, and no preclearing
- **Highly reproducible**—uniform beads help ensure highly 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**—a <40-minute 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**—applicable to IP, Co-IP, and ChIP/RIP assays with detection using western blot or mass spectrometry; ideal for both manual and automated protocols





Benchmarking data: Performance of DynaGreen magnetic beads against competitor magnetic beads



Western blot following either indirect or direct immunoprecipitation workflow to isolate CD3

1. Competitor P Protein A Magnetic Beads
2. Invitrogen™ DynaGreen™ Protein A beads (Thermo Fisher Scientific)
3. Competitor S Protein A Sepharose Beads
4. Competitor T Protein A Magnetic Beads
5. Invitrogen™ Dynabeads™ Protein A beads (Thermo Fisher)
6. DynaGreen Protein A beads (Thermo Fisher)
7. Blank—no sample
8. Competitor S Protein A Sepharose Beads
9. Competitor B Protein A Magnetic Beads

Western blot following either indirect or direct immunoprecipitation workflow to isolate CD81

1. Competitor C Protein A/G Magnetic Beads
2. Thermo Scientific™ CaptureSelect™ IgG-Fc (Multi-species) Magnetic Agarose Beads (Thermo Fisher)
3. Competitor S Protein G Sepharose Beads
4. Competitor T Protein G Magnetic Beads
5. Competitor T Protein A/G Magnetic Beads
6. Invitrogen™ DynaGreen™ CaptureSelect Anti-IgG-Fc (Multi-species) beads (Thermo Fisher)
7. Invitrogen™ DynaGreen™ Protein A/G beads (Thermo Fisher)
8. Competitor G Protein A/G Magnetic Beads
9. Invitrogen™ Dynabeads™ Protein G beads (Thermo Fisher)
10. DynaGreen Protein A/G beads (Thermo Fisher)
11. DynaGreen CaptureSelect Anti-IgG-Fc (Multi-species) beads (Thermo Fisher)
12. Competitor M Protein A/G Magnetic Beads
13. Competitor M Protein G Magnetic Beads
14. Competitor S Protein G Magnetic Sepharose Beads
15. Competitor B Protein G Magnetic Beads

Figure 1. Comparable or better performance with Invitrogen™ Dynabeads™ and DynaGreen™ magnetic beads. Equal amounts of sample material with respect to the antibody and cell lysate were used for all IP protocols according to the manufacturer's protocol. **(A)** We found that Dynabeads Protein A beads and DynaGreen Protein A beads outperformed all competitors in both direct and indirect IP workflows. **(B)** Dynabeads Protein A/G and DynaGreen Protein A/G beads outperformed all competitors in the indirect IP workflow. DynaGreen CaptureSelect Anti-IgG-Fc (Multi-species) beads gave the cleanest IP of CD81, a protein in low abundance, while pulling down a comparable amount of target to Competitor T Protein A/G Magnetic Beads (indirect workflow), Competitor C Protein A/G Magnetic Beads, and Competitor B Protein G Magnetic Beads (direct workflow). Lanes marked with an "M" contain protein marker bands.

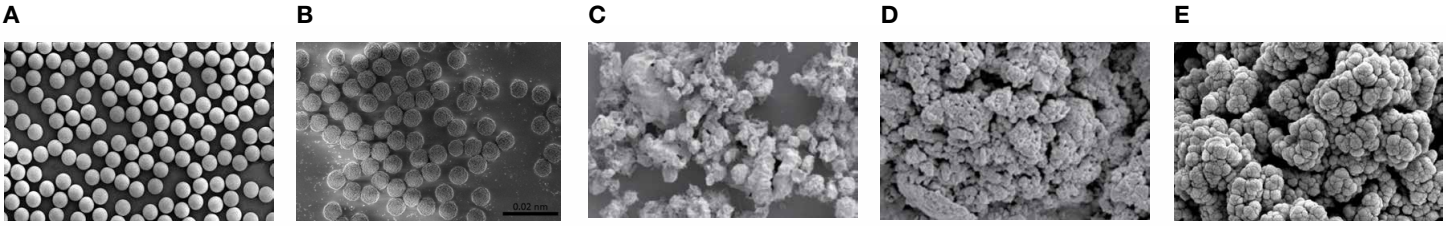


Figure 2. The magnetic bead you choose will affect your results. Dynabeads and DynaGreen magnetic beads have a defined surface to carry out the necessary binding, with no inner surface to trap any unwanted proteins. Dynabeads products **(A)** are the most uniform, monodisperse superparamagnetic beads, manufactured with highly controlled product qualities to help ensure the highest degree of purity and reproducibility, while the DynaGreen products **(B)** offer high purity and higher binding capacity vs. the Dynabeads products, but also offer a more sustainability-conscious version of Dynabeads products. Magnetic particles from alternative suppliers **(C–E)** have variable shapes and sizes that trap impurities, resulting in lower purity and reproducibility.

High-performance protein enrichment for downstream mass spectrometry

Creating a highly pure protein sample with a high yield can be challenging. Protein enrichment, a technique used to concentrate target protein in a sample, is an essential step and is used when isolating low-abundance proteins or reducing the complexity of a sample for proteomic analysis. Enrichment can easily be done through both direct and indirect IP with DynaGreen magnetic beads. In Figure 1A and 1B, we show that high-quality pulldown of both high-abundance (CD3) and low-abundance (CD81) target proteins is easily achieved with DynaGreen magnetic beads at comparable or better performance than today's standard. Furthermore, reproducibility data were gathered in-house through western blotting experiments of five replicates over three batches and shows excellent reproducibility for each of the magnetic beads (Figure 9 on the following pages). To confirm our findings, we commissioned an LC-MS study at the proteomics core facility at the University of Oslo. Findings from these experiments show that target protein was detected in positive controls and not in negative controls. Reproducibility of data was confirmed across batches, as well as verification of reproducibility against a third-party control. Detergents are hard to remove from solution and can, even at very low concentrations, interfere with downstream mass spectrometry (MS) analysis. Contaminations can often be recognized by peak series with equal mass differences at the end of the LC-MS runs. No interfering levels from polymers or surfactants have been reported as part of the external study.

Yes-associated protein (YAP) is a heavily studied co-transcription factor responsible for cancer metastasis, therapeutic resistance, and poor prognosis. It is important to study the binding partners of YAP to better understand metastasis and promote potential therapeutic targets. Dynabeads Protein G magnetic beads are used often in literature to co-immunoprecipitate YAP along with its binding partners to discover new targets for research. In Figures 3, 4, 5, 6, 7, and 8, we demonstrate that Dynabeads Protein G magnetic beads can successfully isolate the target YAP, which is used as a bait to capture TEAD4 and other YAP-binding targets for evaluation with western blot; meeting the need for multiomic studies with a single sample.

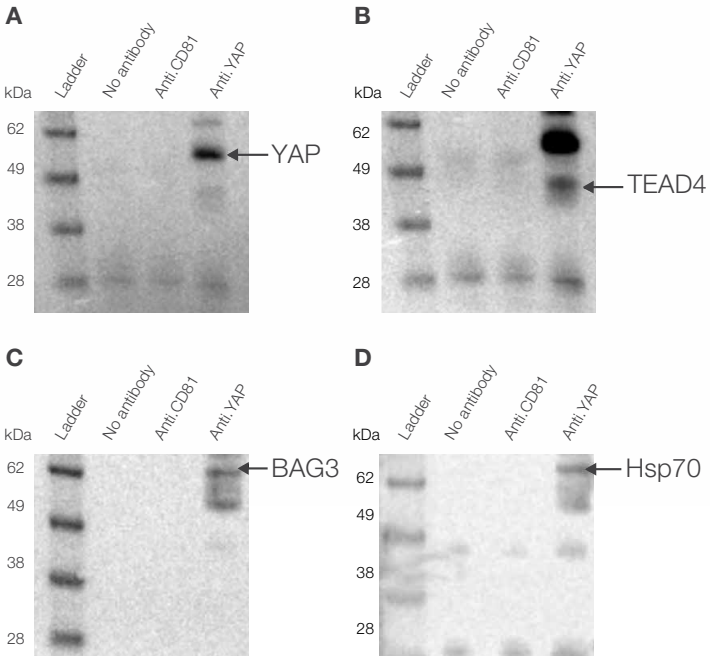


Figure 3. Western blot of Dynabeads Protein G showing co-IP of YAP and its binding targets TEAD4, BAG3, and Hsp70. (A) Successful capture of target protein YAP was only seen after the Dynabeads Protein G were conjugated with anti-YAP antibody. Dynabeads protein G conjugated with anti-CD81 antibody or beads without antibody did not show any binding of target protein YAP. **(B)** Co-IP of successful YAP capture also removed TEAD4 transcription factor. **(C)** BAG3, part of the Hsp70-YAP chaperone complex, was captured after Dynabeads Protein G were conjugated with anti-YAP antibody. **(D)** Through capture of BAG3 by YAP, Hsp70 was also isolated following co-IP of Dynabeads Protein G conjugated with anti-YAP antibody. Dynabeads Protein G conjugated with anti-CD81 antibody or beads without antibody failed to capture YAP and thus did not co-IP TEAD4, BAG3, or Hsp70.

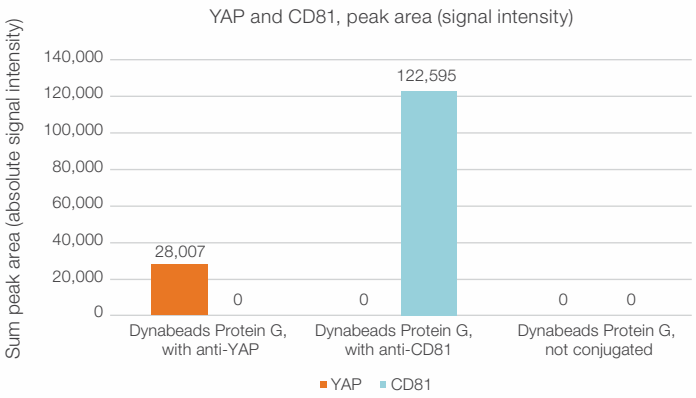


Figure 4. Dynabeads Protein G pulldown of YAP and CD81. Using Dynabeads Protein G conjugated with anti-YAP antibody or anti-CD81 antibody leads to the specific pulldown of the respective target from cell lysate. Nonspecific binding to Dynabeads Protein G without conjugated antibody is low.

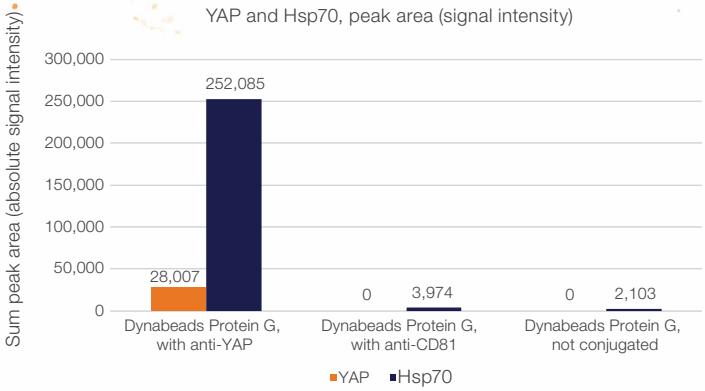


Figure 5. Dynabeads Protein G pulldown of YAP enables co-capture of an interacting partner, Hsp70. Using Dynabeads Protein G conjugated with anti-YAP antibody, Hsp70 is also pulled down from cell lysate. The protocol can be further optimized for co-IP. Nonspecific binding to Dynabeads Protein G conjugated with anti-CD81 antibody or beads without antibody is low.

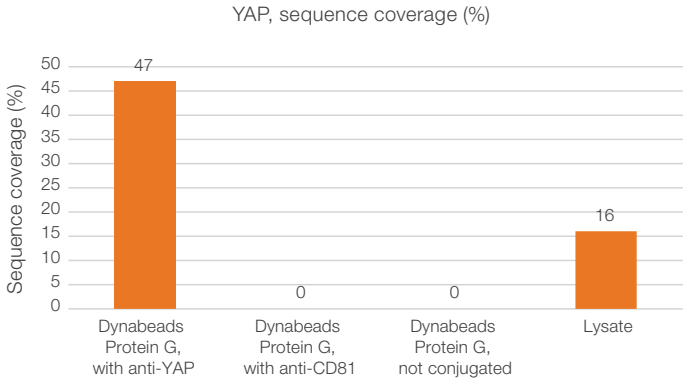


Figure 6. Sequence coverage of YAP. Using Dynabeads Protein G to enrich for the YAP target significantly improves its sequence coverage in the MS dataset, enabling better resolution and improved insight into post-translational modifications.

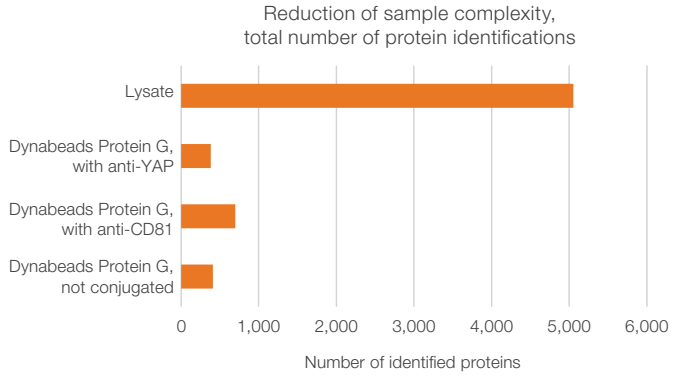


Figure 7. Dynabeads Protein G facilitates the enrichment for proteins of interest, greatly reducing sample complexity and enabling better resolution of low-abundance proteins. 90% of the sample complexity is reduced using Dynabeads Protein G compared to the full cell lysate.

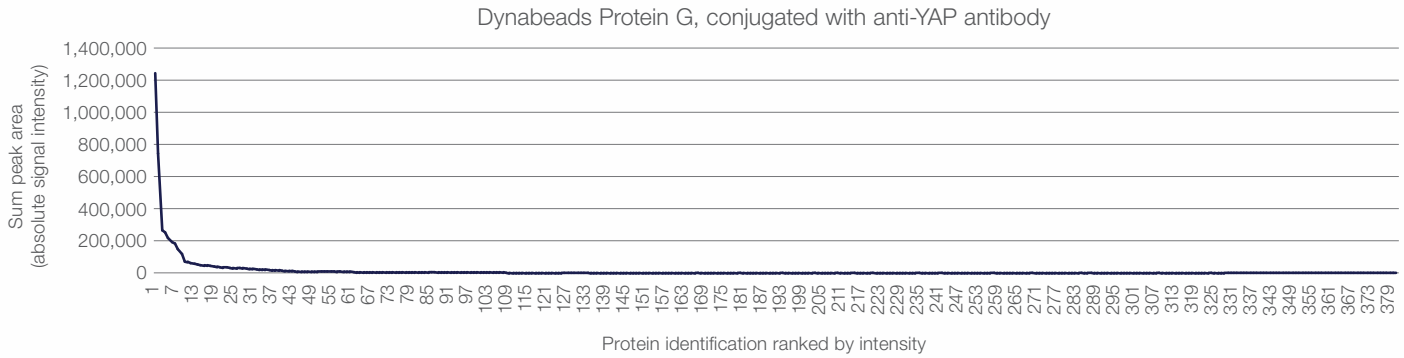


Figure 8. Among the 379 proteins pulled down from cell lysate using Dynabeads Protein G conjugated with anti-YAP antibody, the majority of signal comes either from the target itself, its binding partners, or the common contaminants in an MS workflow. Very few proteins are captured besides the target. A large number of proteins identified with very low intensity are nonspecific binders at very low levels. These nonspecific binders are quickly identified and typically rejected as potential binding partners during data analysis.

Choose high-performing magnetic beads without microplastics for immunoprecipitation

IP with DynaGreen magnetic beads

DynaGreen magnetic beads are a highly magnetic, submicron bead platform with a pioneering sustainable design, from manufacture to customer site.

Leading with DynaGreen Protein A, DynaGreen Protein A/G, and DynaGreen CaptureSelect Anti-IgG-Fc (Multi-species) beads, these 250 nm superparamagnetic beads enable high-performance direct and indirect IP of proteins, protein complexes, protein–nucleic acid complexes, and other antigens (Ag).

The magnetic separation technology used by DynaGreen magnetic beads is rapid and gentle, thereby causing minimal physical stress to your target proteins. The beads, consisting of a nonmicroplastic bead core and made with biosolvents, give reproducible results with low nonspecific binding and are perfectly suitable for downstream mass spectrometry and western workflows.

The DynaGreen CaptureSelect Anti-IgG-Fc (Multi-species) beads have also shown promising results for affinity-based isolation of exosomes from liquid biopsy samples within 40 minutes (data not shown).

The submicron bead size increases the available target capture surface area, resulting in efficient isolation of target protein in less than 80 minutes via a simple bind-incubate-elute IP protocol that does not require preclearing.

DynaGreen magnetic beads can also facilitate automation of IP with any Thermo Scientific™ KingFisher™ sample purification system in a simple 40-minute protocol that greatly reduces hands-on time and manual errors.

Highlights:

- **High performance**, **yield**, and **purity** for direct and indirect IP
- **Sustainable** and holistic product design with **energy-efficient** manufacturing, **recyclable** packaging, and a **nonmicroplastic** magnetic bead core
- Backed by 30 years of **Dynabeads magnetic beads quality** and innovation
- **Automated workflow** on KingFisher systems

DynaGreen Protein A, Protein A/G and CaptureSelect Anti-IgG-FC reproducibility

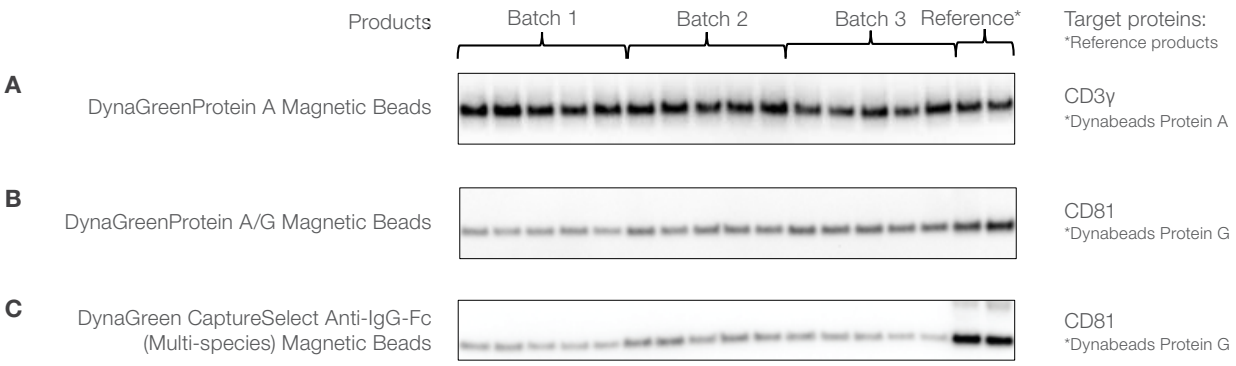


Figure 9. Excellent reproducibility of DynaGreen magnetic beads. Samples from five independent IP experiments at 0.5 mg for DynaGreen Protein A **(A)**, DynaGreen Protein A/G **(B),** and DynaGreen CaptureSelect Anti-IgG-Fc **(C)** beads, were analyzed. Two replicates of a Dynabeads Protein A or Dynabeads Protein G were used as controls. CD3γ: observed size 21–25 kDa. CD81: observed size ~26 kDa. The experiments show that at least three out of five parallel target bands appear with similar intensity, judged by visual inspection. All target bands are visualized with the same molecular weight.

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 want a generic product you can use to isolate other ligands, such as nucleic acids, cells, and exosomes

Choose (3) if you have a recombinant protein (fusion tag)—our most popular fusion tags for recombinant protein expression are covered by magnetic beads.

Choose this if you have	Surface coating on the magnetic beads	Type of ligand required	Nonspecific binding	Main benefits for IP	Products
(1) Antibody that recognizes your protein	Protein A, G, or A/G	Primary antibodies from most species. Protein A and G bind different antibody species and subclasses with different specificities.	Low	• Fastest, easiest protocol • Low nonspecific binding	Dynabeads Protein A Dynabeads Protein G Immunoprecipitation Kit Dynabeads Protein A Immunoprecipitation Kit Dynabeads Protein G DynaGreen Protein A DynaGreen Protein A/G
	Secondary or recombinant antibodies	Multispecies IgGs	Low	• Fast and easy protocol • Low nonspecific binding • Flexible, multispecies IgG binding with the CaptureSelect IgG-Fc (ms) beads	Dynabeads M-280 Sheep anti-Mouse IgG Dynabeads M-280 Sheep anti-Rabbit IgG DynaGreen CaptureSelect Anti-IgG-Fc (Multi-species)
	Epoxy–coated beads*	Any protein ligand (e.g., antibody, peptide)	Ultralow	• 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	Low	• Binds any biotinylated protein • For samples high in soluble IgGs • Recombinant Ab lacking the Fc-region	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)	Low	• Purify many different proteins incorporated with the same tag • No need for antibodies	See thermofisher.com/iptags for product overview

* See more choices in surface-activated Dynabeads products for the binding and capture of additional targets.

Manual vs. automated IP

The intrinsic features of our magnetic beads for IP make them ideally suited for automation. The beads disperse well, sediment slowly, and move quickly with an even pull to the magnet. This facilitates rapid target binding and short incubation and separation times. Thus, combining our magnetic beads for IP with KingFisher systems facilitates higher throughput, reduces hands-on time, and secures reproducibility.

	Manual IP	Automated IP
Incubate, wash, and elute	35 minutes	13 minutes
Western blot	60 minutes	7 minutes
Immunolabeling	2 days	7 hours

Co-IP: With Dynabeads and DynaGreen products, you can help to ensure that intact protein complexes are isolated and remain intact

If you are using nonmagnetic bead–based 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 and DynaGreen 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

Four 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 and DynaGreen magnetic beads because all antibodies are accessible on the smooth bead surface, limiting nonspecific background.
Preclearing is necessary to get good IP results.	A preclearing step is unnecessary with Dynabeads and DynaGreen magnetic beads. You can save time and use half the amount of solid phase, which helps save money.
Higher capacity is better for IP.	Alternative magnetic beads on the market claiming high binding capacity binds a lot of precious antibody and thus increases the total cost while also increasing the unspecific binding of proteins. Even with a lot of washing, you will end up with unwanted background. Dynabeads and DynaGreen magnetic beads give a good IP yield with high purity.
Dynabeads magnetic beads are expensive.	With no preclearing and less antibody used, Dynabeads and DynaGreen 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
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
DynaGreen Protein A	3 mL	80102G
DynaGreen Protein A/G	3 mL	80105G
DynaGreen CaptureSelect Anti-IgG-Fc (Multi-species)	3 mL	80108G

* Most products are available in larger pack sizes.

Resources:

More information: thermofisher.com/immunoprecipitation
thermofisher.com/kingfisher

FAQs: thermofisher.com/ipfaqs

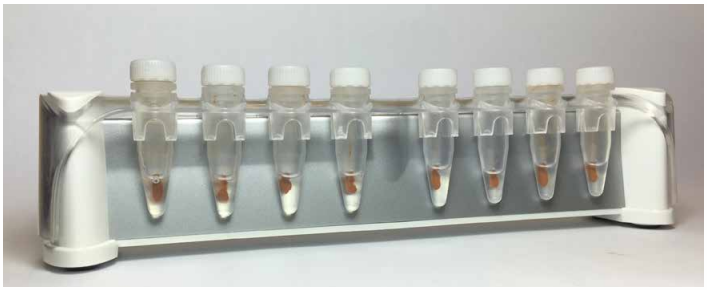
Fusion tags: thermofisher.com/iptags

Videos: youtube.com/immunoprecipitation
Immunoprecipitation myth videos
Immunoprecipitation publication trends—the reasons for the shift
Automated immunoprecipitation in 40 min using Dynabeads and KingFisher Flex products

Thermo Scientific™ Pierce™ magnetic beads: thermofisher.com/proteinpurification

Magnetic stands

Invitrogen™ DynaMag™ 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 Invitrogen™ DynaMag™-2 Magnet.

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

Extra rack for DynaMag-2 Magnet

The DynaMag Plastic Rack can be purchased as a separate unit to increase the flexibility of the magnet use.

- Holds up to 16 standard 1.5 mL or 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 helps ensure equal vortexing of all tubes
- Efficient control and visibility of your proteins and nucleic acids isolations

DynaMag-Spin Magnet

Other magnets available for immunoprecipitation are:

Invitrogen™ DynaMag™-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.

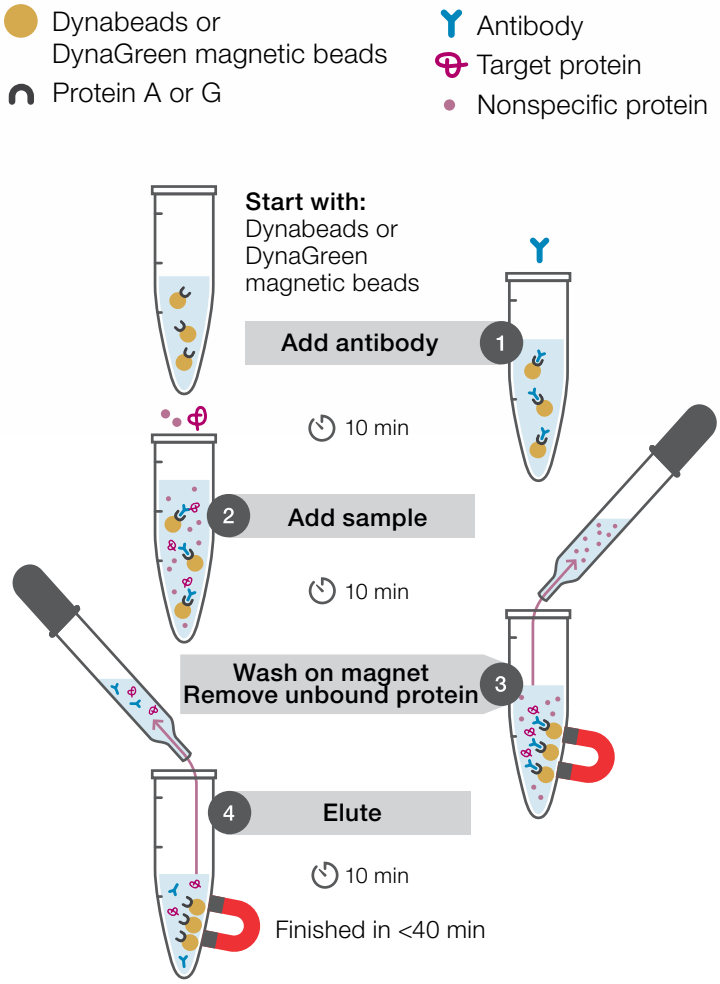
Plate magnets

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

Resources and ordering information for magnetic stands

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

Get more information at thermofisher.com/magnets



"Dynabeads Protein G beads 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."

Figure 10. IP in less than 40 minutes. Dynabeads and DynaGreen 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.

Find out more at **thermofisher.com/immunoprecipitation**