Protein biology

High-performance, environmentally sustainable magnetic beads for immunoprecipitation to facilitate proteomics

DynaGreen magnetic beads—pioneering environmentally sustainable product design

Keywords

DynaGreen, immunoprecipitation, mass spectrometry, western blot, sustainable product design, green chemistry, submicron, microplastic-free, magnetic beads, KingFisher sample purification systems, automation

In this application note, we present:

- A unique microplastic-free submicron magnetic bead
- A pioneering sustainable product design
- A high-performance toolbox for proteomics research
- A simple, fast, flexible, and reliable method for isolating target proteins
- Target protein yield and purity compatible with western blots and mass spectrometry
- A workflow that can seamlessly scale from manual to automated handling

Introduction

Proteomics research is a dynamically growing field, enabling direct discovery of protein targets for novel diagnostic, prognostic, or therapeutic purposes. One of the key challenges in proteomics research, due to the high degree of complexity and dynamic range of the proteome, is to detect a protein with optimal sensitivity. Immunoprecipitation (IP) directly addresses this limitation by enriching specific target proteins or protein complexes and thereby reducing sample complexity. At the same time, researchers and funding bodies are becoming increasingly aware of the impact that research practices have on the environment. This application note presents Invitrogen™ DynaGreen™ magnetic beads—a new, high-performing, and environmentally sustainable IP option to facilitate progress in proteomics research. This advanced IP solution is a microplastic-free magnetic bead platform that was designed with sustainability in mind—from production to the hands of the researcher—by implementing the twelve principles of green chemistry [1]. Furthermore, DynaGreen magnetic beads have ACT™ labels. These are third party–verified environmental impact scores published by the My Green Lab™ organization, which enable researchers to make informed choices on sustainable products.

A toolbox for fast and simple high-purity immunoprecipitation

IP is a method that allows isolation of a target protein or protein complexes for downstream analysis. As protein complexes often involve transient and weak interactions, it is critical to use a method that offers rapid binding kinetics and low nonspecific binding, in order to reproducibly isolate a specific target or target complexes.

Here we present an IP toolbox comprising Invitrogen™ DynaGreen™ Protein A, DynaGreen™ Protein A/G, and DynaGreen™ CaptureSelect™ Anti-IgG-Fc (Multi-species) magnetic beads. These products offer a choice of a surface ligand that enables capture of antibodies from a wide range of species and isotypes, providing significant flexibility to the field of proteomic research. DynaGreen magnetic beads are designed for high-performance IP and are engineered for seamless incorporation into existing magnetic bead–based IP protocols, facilitating an easy transition from alternative magnetic beads to a more environmentally sustainable option.

With their unique submicron size, DynaGreen magnetic beads (Figure 1) also provide rapid binding kinetics (Figure 2). The proximity of the beads to the target in the solution translates to short incubation times and therefore fast protocols of less than 80 minutes. Additionally, the same IP protocol can be effortlessly scaled to increase sample throughput and reduce protocol time by using the readily available, automated DynaGreen magnetic bead IP protocols for the Thermo Scientific™ KingFisher™ instruments (Figure 3).

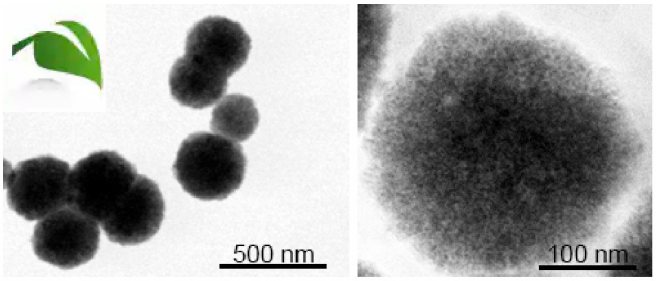

Figure 1. Scanning transmission electron microscopy (STEM) image of DynaGreen magnetic beads.

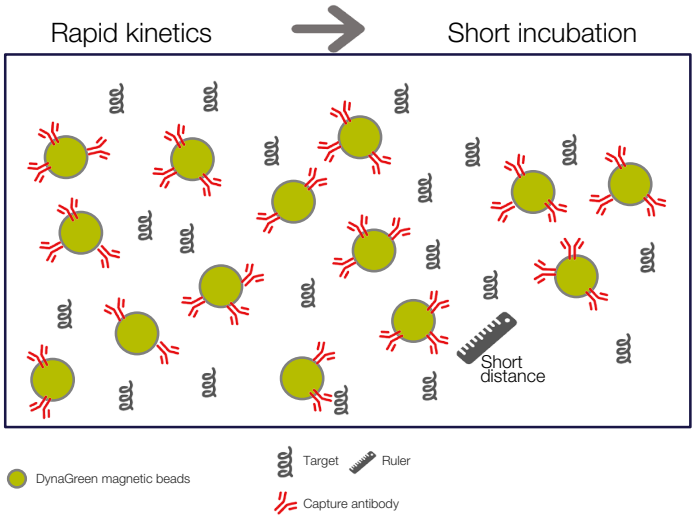

Figure 2. Binding kinetics of DynaGreen magnetic beads. The submicron beads have a collectively larger surface area for capturing target protein. Together with their proximity to targets while in suspension, this results in rapid binding kinetics and fast isolation.

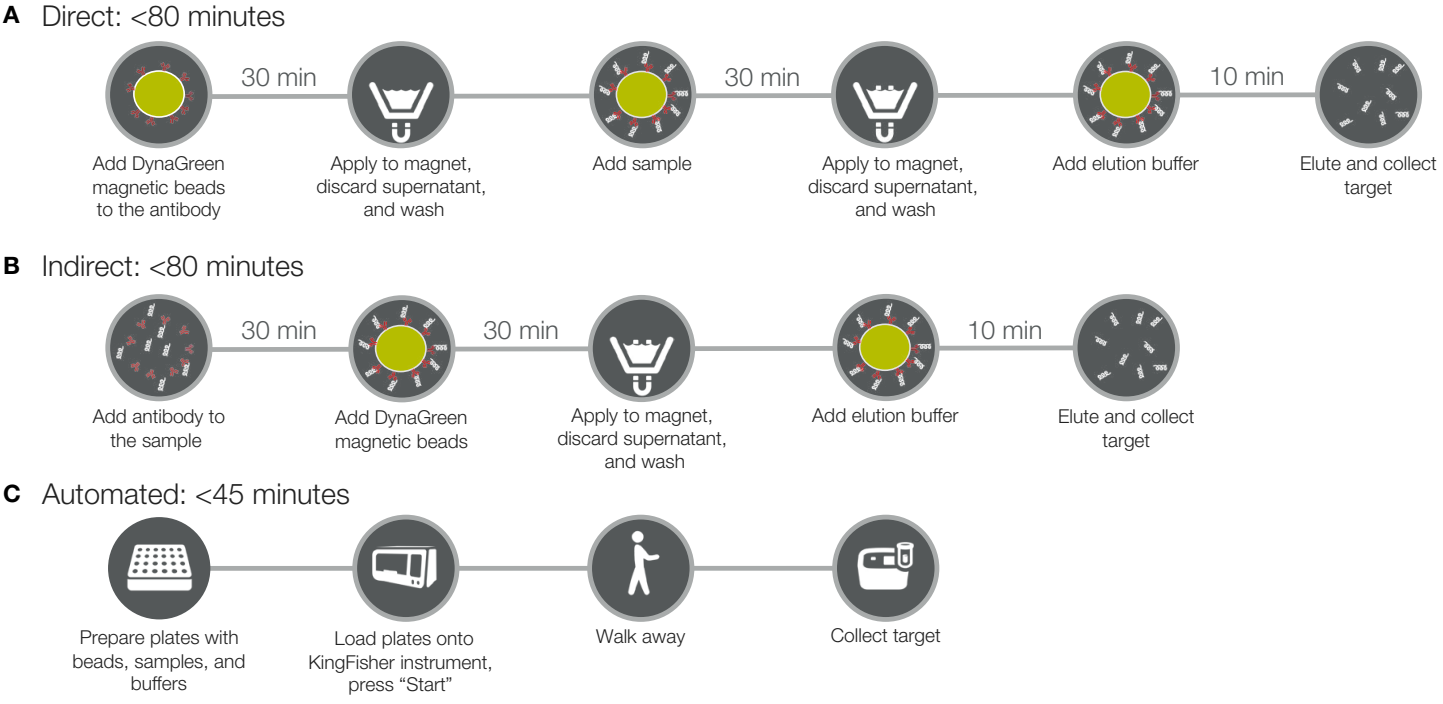

Figure 3. Overview of (A) manual direct, (B) manual indirect, and (C) automated IP workflows using the DynaGreen magnetic beads.

The automated protocol using the KingFisher instruments can be run in just 40 minutes with up to 96 samples per run, which significantly reduces hands-on time while maintaining the same reproducible high target yield and low nonspecific binding as obtained by the manual process (Figure 3). Short protocols are especially important when capturing weak and transient interactions between different proteins (co-IP) and to obtain superior signal-to-noise ratios.

DynaGreen magnetic beads, a pioneering, environmentally sustainable IP solution, facilitate:

- Rapid and efficient target binding for minimized IP protocol time
- Reproducible high-yield and high-purity target capture
- Target enrichment compatible with downstream western blotting and mass spectrometry
- Low antibody consumption due to nonporous nature of the beads
- Low consumption of beads per assay due to efficient binding kinetics of submicron beads
- Reduced environmental impact of research without compromising results

Low nonspecific binding for high-purity target capture

The unique surface properties and fast binding kinetics offered by the innovative product design help ensure very low nonspecific binding (Figure 4). The three types of DynaGreen beads were functionalized with antibodies against CD3 or CD81 and exposed to cell lysates, according to the IP protocol (Figure 3), followed by electrophoresis and finally Coomassie staining. All three products demonstrated very low background and performance similar to Dynabeads magnetic beads used as a positive control (Figure 4).

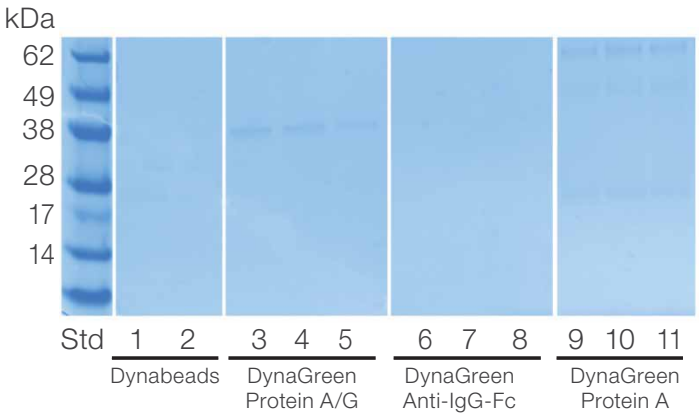

Figure 4. IP of CD81 and CD3 from Jurkat cell lysate with the three types of DynaGreen beads, followed by Coomassie staining of gels. Invitrogen™ Dynabeads™ magnetic beads serve as the positive control.

Flexibility of direct or indirect IP approach for reproducible target recovery

DynaGreen magnetic beads were developed to provide high-performance IP via either a direct or indirect IP workflow to enable efficient capture over a broad range of target abundance.

The direct IP protocol starts with addition of the primary antibody to the DynaGreen magnetic beads, followed by an incubation step. Unbound antibodies are washed off. The functionalized DynaGreen magnetic beads are then incubated with the target-containing sample. Prior to elution of the target, wash steps are included to remove unbound protein and minimize nonspecific binding (Figure 3).

For low-abundance target proteins or when the antibody has a weak binding affinity towards the antigen, an indirect IP protocol can be performed. In this case, primary antibodies are added to the sample and incubated. The DynaGreen magnetic beads are then added to pull out the antibody bound to the target from the sample (Figure 3).

Figure 5 shows how species- and isotype-specific antibodies compatible with corresponding antibody-binding proteins on the DynaGreen surface perform in immunoprecipitation. DynaGreen Protein A/G and DynaGreen CaptureSelect Anti-IgG-Fc (Multi-species) beads were functionalized with mouse IgG1 anti-CD81 antibodies, while DynaGreen Protein A beads were functionalized with rabbit IgG anti-CD3 antibodies. The functionalized DynaGreen beads were incubated with Jurkat cell lysate according to the IP protocol. After incubation, CD81 and CD3 were eluted with Invitrogen™ NuPAGE™ LDS Sample Buffer or low pH. All three DynaGreen products provided target recovery in a reproducible manner and demonstrated compatibility with both denaturing and low-pH elution buffers.

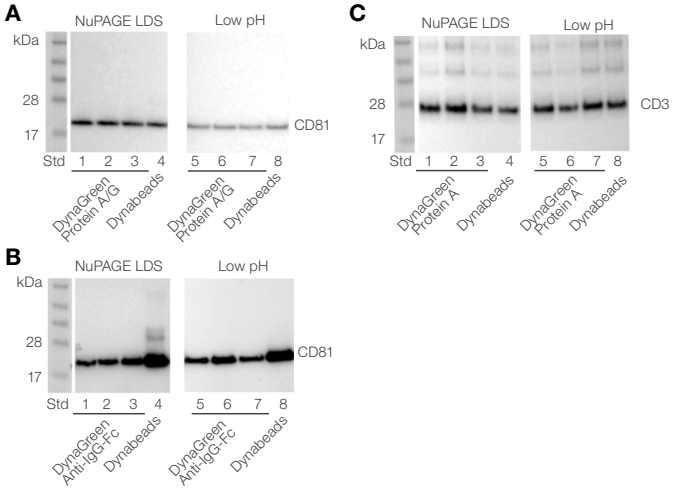

Figure 5. IP and western blot analysis of isotype-specific antibodies. (A, B) IP of CD81 with DynaGreen Protein A/G and DynaGreen CaptureSelect Anti-IgG-Fc (Multi-species) beads, and **(C)** IP of CD3 with DynaGreen Protein A beads. After incubation, elution was carried out with LDS buffer or low pH. Dynabeads magnetic beads were used as the positive control.

When systematically compared to a broad range of magnetic beads from alternative suppliers, DynaGreen beads demonstrated equal or higher target yield and/or purity (Figure 6). DynaGreen beads also showed excellent performance in both direct and indirect workflows, thereby offering researchers increased flexibility to tune the workflow according to the target proteins.

Demonstrated compatibility with mass spectrometry analysis

DynaGreen magnetic beads functionalized with anti-CD81 or anti-CD3, as described in Figure 5, were successfully used to isolate CD81 and CD3 for downstream mass spectrometry (Table 1). Three analytical replicates of each DynaGreen product were used to isolate target proteins.

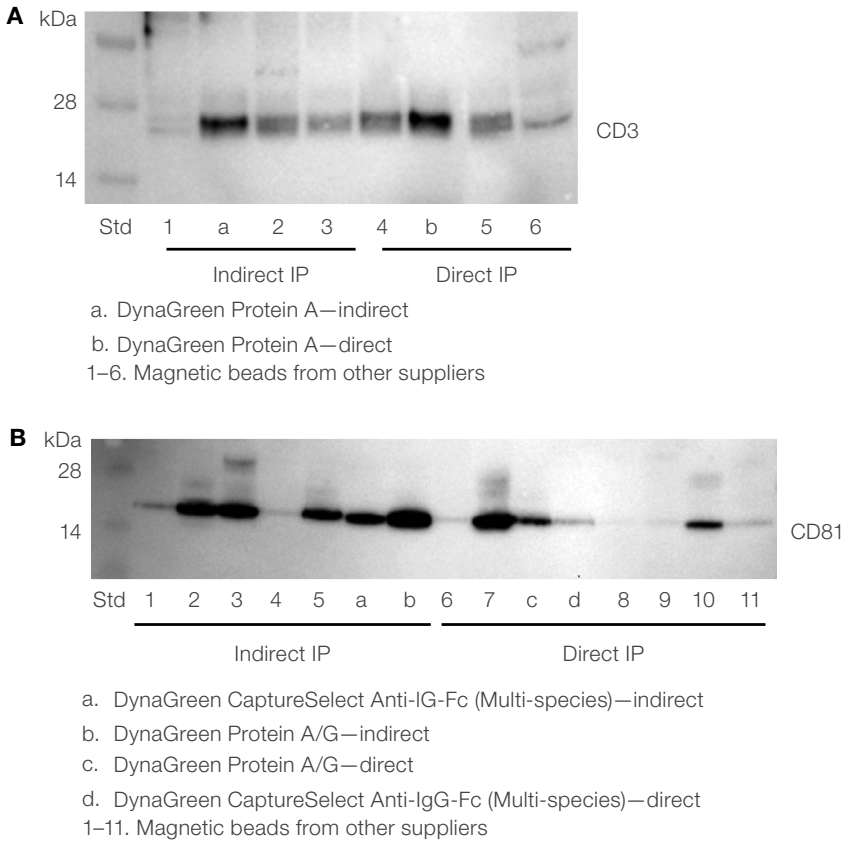

Figure 6. IP of CD81 or CD3 from Jurkat cell lysate using a direct or indirect protocol with DynaGreen products or other magnetic beads providers, followed by western blot analysis. **(A)** IP of CD3 with DynaGreen Protein A (a, b) and other suppliers' magnetic beads. **(B)** IP of CD81 with DynaGreen Protein A/G (b, d), DynaGreen CaptureSelect Anti-IgG-Fc (Multi-species) (a,c) or other suppliers' magnetic beads.

Table 1. Mass spectrometry summary of IP using functionalized DynaGreen magnetic beads. Three analytical replicates of each DynaGreen product were used to isolate the target protein. The table shows counts of target-specific peptides and signal intensity in IP samples and in negative-control samples without primary antibody. Dynabeads Protein G and Dynabeads Protein A are used as controls. TIC: total ion current.

DynaGreen Protein A/G: target = CD81

	Readout	Rep 1	Rep 2	Rep 3	Dynabeads Protein G
With antibody	TIC	2,469,761	2,408,487	2,743,954	2,059,571
	Number of target peptides	5	6	4	4
Without antibody	TIC	–	–	34381	–
	Number of target peptides	–	–	2	–

DynaGreen CaptureSelect Anti-IgG-Fc (Multi-species): target = CD81

	Readout	Rep 1	Rep 2	Rep 3	Dynabeads Protein G
With antibody	TIC	3,146,538	3,980,040	2,961,177	2,059,571
	Number of target peptides	4	4	4	4
Without antibody	TIC	–	–	–	–
	Number of target peptides	–	–	–	–

DynaGreen Protein A: target = CD3γ

	Readout	Rep 1	Rep 2	Rep 3	Dynabeads Protein A
With antibody	TIC	4,737,774	3,897,153	4,858,358	2,768,378
	Number of target peptides	11	10	10	8
Without antibody	TIC	–	–	–	–
	Number of target peptides	–	–	–	–

The target proteins were analyzed by mass spectrometry, and results are presented as number of identified target-specific peptides as well as signal intensity (TIC). The numbers of identified target peptides were similar to or higher than those obtained with Dynabeads control beads. Furthermore, no interfering levels of polymers or surfactants were reported, and no target protein was present in the negative-control samples (immunoprecipitated without a primary antibody). For DynaGreen Protein A/G beads, the target peptide was detected in one of the four negative-control samples, but at ~0.01x the signal intensity of the positive-control sample (most likely due to targeted acquisition and nonspecific binding).

Summary

Here we have described a simple, rapid, and reliable bead-based method for IP. The method takes advantage of unique submicron-sized DynaGreen magnetic beads with an innovative sustainable product design. The high-performing, versatile DynaGreen magnetic beads for rapid target isolation were designed with sustainability in mind, from development to manufacturing and shipping. Principles of green chemistry were applied at each development step, leading to a product that facilitates proteomics research in a more environmentally friendly way. At the same time, these magnetic beads do not compromise on quality, offering great target yield and low nonspecific binding for both western and mass spectrometry applications.

Reference

1. *Green Chemistry: Theory and Practice.* Anastas and Warner, Oxford University Press, 1998.

Ordering information

Product	Quantity	No. of reactions	Cat. No.
DynaGreen Protein A Magnetic Beads	0.5 mL	20	80101G
	3 mL	120	80102G
	25 mL	1,000	80103G
DynaGreen Protein A/G Magnetic Beads	0.5 mL	20	80104G
	3 mL	120	80105G
	25 mL	1,000	80106G
DynaGreen CaptureSelect Anti-IgG-Fc (Multi-species) Magnetic Beads	0.5 mL	20	80107G
	3 mL	120	80108G
	25 mL	1,000	80109G
DynaMag-2 Magnet	1 each	–	12321D
KingFisher Apex Purification System			5400910

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