DynaGreen™ Protein A, DynaGreen™ Protein A/G, and DynaGreen™ CaptureSelect™ Anti-IgG-Fc (Multi-species)

Manual protocols for direct or indirect immunoprecipitation

Catalog Numbers 80101G, 80102G, 80103G, 80104G, 80105G, 80106G, 80107G, 80108G, and 80109G

Pub. No. MAN0028533 **Rev.** C.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

DynaGreen™ Protein A, DynaGreen™ Protein A/G, and DynaGreen™ CaptureSelect™ Anti-IgG-Fc (Multi-species) magnetic beads are designed for immunoprecipitation of proteins, protein complexes, protein-nucleic acid complexes, and other antigens. DynaGreen™ magnetic beads can be used for both indirect and direct immunoprecipitation. Target-specific antibodies bind to the DynaGreen™ magnetic bead surface through their Fc regions. The unique magnetic properties of the DynaGreen™ magnetic beads allow for easy collection of target and removal of supernatant.

DynaGreen™ magnetic beads are provided at a concentration of 20 mg/mL and provide approximately 40 reactions per mL. Please note that there is a natural variation of the color of individual bead batches, ranging from black to golden brown.

Contents and storage

Product	Cat. No.	Amount	Number of reactions
DynaGreen™ Protein A magnetic beads[1]	80101G	0.5 mL	20
	80102G	3 mL	120
	80103G	25 mL	1000
DynaGreen™ Protein A/G magnetic beads[1]	80104G	0.5 mL	20
	80105G	3 mL	120
	80106G	25 mL	1000
DynaGreen™ CaptureSelect™ Anti-IgG-Fc (ms) magnetic beads	80107G	0.5 mL	20
	80108G	3 mL	120
	80109G	25 mL	1000

[1] Contains 20 mg/mL of beads in phosphate buffered saline (PBS), pH 7.4, with a biodegradable surfactant and a preservative.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Catalog numbers that appear as links open the web pages for those products.

Item	Source
1.5 mL microcentrifuge tubes	MLS
Antibody for immunoprecipitation	MLS
DynaMag™ Magnet	12321D
Sample mixer (e.g. HulaMixer™ Sample Mixer)[1]	15920D
RIPA Lysis and Extraction Buffer	89900
Phosphate-buffered saline (PBS), pH 7.4 [2]	10010023
Glycine (50 mM), pH 2.8	MLS
NuPAGE™ LDS Sample Buffer	NP0007
NuPAGE™ Sample Reducing Agent	NP0009

[1] Sample mixer needs to allow tilting and rotation of tubes
[2] Prepare PBS with and without 0.05% Tween™ 20

Procedural guidelines

- The amount of antibody (Ab) captured by DynaGreen™ magnetic beads depends on the concentration of beads and Ab in the starting sample, as well as the type of immunoglobulin being bound (see “Affinity” on page 6).
- For standard immunoprecipitation, use PBS for antibody binding and washing steps. Other possible buffers include alternative phosphate buffers, HEPES, Tris, and lysis buffer (e.g. RIPA, NP40). Elution buffer may also be substituted by alternative low pH, high pH, or high salt buffers, depending on the application.
- An incubation time of 30 minutes for immunoprecipitation is sufficient for most applications. Increasing the incubation time up to 120 minutes can increase yield, particularly for low affinity Abs, but may increase non-specific binding.
- For sensitive proteins and phosphorylation studies, perform the isolation protocol and elution at 2–8°C to avoid protein complex dissociation and minimize enzymatic activity. Add proteases and/or phosphatase inhibitors during the lysis to avoid protein degradation.
- For mass spectrometry (MS), increase the amount of starting material, beads, and Ab. Increase the binding time of the antibody of the beads to 1 hour. Optimization may be required for each Ab and target antigen. Increasing number of washes at the end of the procedure and washing with more stringent reagents might decrease non-specific background. Use PBS without Tween™ 20 in the last 2 washes to remove detergent before MS. Beads and protocol-recommended buffers are compatible with on-bead peptide digestion.

Before you begin

- This protocol provides a general procedure for immunoprecipitation. Optimization may be required for each antibody and target antigen. The protocol uses 25 µL of DynaGreen™ Protein A, DynaGreen™ Protein A/G magnetic beads, or DynaGreen™ CaptureSelect™ Anti-IgG-Fc (ms), but may be scaled up or down as required.

  IMPORTANT! It is essential to ensure thorough mixing of the beads with the buffers, Ab, and antigen to obtain the desired result.

- Cells may be lysed using any standard cell lysis protocol compatible with your starting material. We recommend the use of Pierce™ IP Lysis Buffer. For protocols and additional information about cell lysis, see **thermofisher.com/immunoprecipitation**.

Direct immunoprecipitation

Prepare DynaGreen™ magnetic beads

1. To resuspend DynaGreen™ magnetic beads in the vial, vortex >30 seconds or tilt and rotate 5 minutes until visually resuspended.
2. Transfer 25 µL (0.5 mg) of DynaGreen™ magnetic beads to a fresh tube.
3. Place the tube on the magnet to separate the beads from the solution, then remove the supernatant.
4. Remove the tube from the magnet.
5. Proceed directly to “Bind antibody”.

Bind antibody

1. Add your Ab (typically 1–10 µg), diluted in 200 µL PBS with Tween™ 20, to the DynaGreen™ magnetic beads from step 4. Mix by gentle pipetting.

   Note: The optimal amount of Ab depends upon the individual Ab used.
2. Incubate with rotation for 30 minutes at room temperature.
3. Place the tube on the magnet, then remove the supernatant.
4. Remove the tube from the magnet, then resuspend the magnetic bead-Ab complex in 200 µL PBS with Tween™ 20. Wash by gentle pipetting.
5. Proceed to “Immunoprecipitate target antigen”.

Immunoprecipitate target antigen

1. Place the tube (see step 4) on the magnet, then remove the supernatant.
2. Add your sample containing the antigen (200 µL), then gently pipette to resuspend the magnetic bead-Ab complex.
3. Incubate with rotation for 30 minutes at room temperature to allow antigen to bind to the magnetic bead-Ab complex.
4. Place the tube on the magnet.
5. Wash the magnetic bead-Ab-antigen complex 3 times by gentle pipetting using 200 µL of either PBS- Tween™ 20 (for non-denaturing elution) or PBS with or without Tween™ 20 for denaturing elution in LDS. For each wash, place tube on the magnet and remove the supernatant, then remove tube from the magnet, add wash buffer and resuspend by gentle pipetting.
6. Proceed to “Elute target antigen”.

Elute target antigen

Elution in LDS buffer

1. Place the tube (see step 5) on the magnet, then remove the supernatant.
2. Add 25 µL of NuPAGE™ LDS Sample Buffer diluted to 1X in water.
3. Gently pipette to resuspend the magnetic bead-Ab-antigen complex.
4. Heat for 10 minutes at 70°C with mixing.
5. Place the tube on the magnet, then load the bead-free supernatant/sample onto a gel.

Non-denaturing elution

1. Place the tube (see step 5) on the magnet, then remove the supernatant.
2. Spin down the beads and any remaining wash buffer for 5 seconds, place tube on the magnet, then thoroughly remove the supernatant.
3. Add 20 µL glycine buffer, then gently pipette to resuspend the magnetic bead Ab-antigen complex.

   Note: Avoid foaming.
4. Incubate with mixing for 10 minutes at room temperature to dissociate the magnetic bead Ab-antigen complex.
5. Place the tube on the magnet, then transfer the supernatant containing eluted Ab and antigen to a clean tube.

   Note: If the eluted protein is to be used for functional assays or stored, the pH of the eluate can be neutralized by adding e.g. 1 M Tris, pH 7.5.

Indirect immunoprecipitation

Bind antibody to the target

1. Add your antibody (typically 1–10 µg), directly into the cell lysate (200 µL). Mix by gentle pipetting.
2. Incubate with rotation for 30 minutes at room temperature.
3. During incubation, proceed to “Prepare DynaGreen™ magnetic beads”.
4. After incubation, proceed to “Immunoprecipitate target antigen”.

Prepare DynaGreen™ magnetic beads

1. Resuspend DynaGreen™ magnetic beads in the vial, then vortex >30 seconds or tilt and rotate 5 minutes until visually resuspended.
2. Transfer 25 µL (0.5 mg) of DynaGreen™ magnetic beads to a tube.
3. Place the tube on the magnet to separate the beads from the solution, then remove the supernatant.
4. Remove the tube from the magnet.

Immunoprecipitate target antigen

1. To your beads, add your sample containing the antigen bound to the Ab (approximately 200 µL), then gently pipette to resuspend the magnetic beads.
2. Incubate with rotation for 30 minutes at room temperature to allow the Ab-antigen complexes to bind to the magnetic beads.
3. Place the tube on the magnet.
4. Wash the magnetic bead-Ab-antigen complex 3 times by gentle pipetting using 200 µL of either PBS- Tween™ 20 (for non-denaturing elution) or PBS with or without Tween™ 20 for denaturing elution in LDS. For each wash, place tube on the magnet and remove the supernatant, then remove tube from the magnet, add wash buffer and resuspend by gentle pipetting.
5. Proceed to “Elute target antigen”.

Elute target antigen

Elution in LDS buffer

1. Place the tube (see step 4) on the magnet, then remove the supernatant.
2. Add 25 µL of NuPAGE™ LDS Sample Buffer diluted to 1X in water.
3. Gently pipette to resuspend the magnetic bead-Ab-antigen complex.
4. Heat for 10 minutes at 70°C with mixing.
5. Place the tube on the magnet, then load the bead-free supernatant/sample onto a gel.

Non-denaturing elution

1. Place the tube (see step 4) on the magnet, then remove the supernatant.
2. Spin down the beads and any remaining wash buffer for 5 seconds, place tube on the magnet, then thoroughly remove the supernatant.
3. Add 20 µL glycine buffer, then gently pipette to resuspend the magnetic bead Ab-antigen complex.

   Note: Avoid foaming.
4. Incubate with shaking for 10 minutes at room temperature to dissociate the magnetic bead Ab-antigen complex.
5. Place the tube on the magnet, then transfer the supernatant containing eluted Ab and antigen to a clean tube.

   Note: If the eluted protein is to be used for functional assays or stored, the pH of the eluate can be neutralized by adding e.g. 1 M Tris, pH 7.5.

Affinity

Table 1 Affinity of proteins coupled to DynaGreen™ for the different antibody subclasses

Species	Antibody class	Affinity for protein A	Affinity for protein A/G	Affinity for anti-IgG-Fc
Human	IgG1	+++	+++	+++
	IgG2	+++	+++	+++
	IgG3	+	+++	+++
	IgG4	+++	+++	+++
	IgM	+	+	–
	IgA	+	+	–
	IgD	–	–	–
Mouse	IgG1	+	+++	+++
	IgG2a	+++	+++	+++
	IgG2b	+++	+++	+++
	IgG3	+++	+++	+++
	IgM	+	–	Not tested
Rat	IgG1	+	++	B[1]
	IgG2a	–	+++	B
	IgG2b	–	+	B
	IgG2c	+++	+++	B
	IgG	+++	+++	+++
Rabbit	IgG	+++	+++	+++
Chicken	IgY	–	–	–
Cow	IgG1	+	+++	B
	IgG2	+++	+++	B
Goat	IgG1	+	+++	Not tested
	IgG2	+++	+++	Not tested
Monkey	IgG	+++	+++	Not tested
Sheep	IgG1	+	+++	Not tested
	IgG2	+++	+++	Not tested
Dog	IgG	+++	+++	Not tested
Hamster	IgG	+++	+++	Not tested
Guinea Pig	IgG	+++	+++	Not tested
Pig	IgG	+++	+++	Not tested

[1] B= Binding

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Troubleshooting

Observation	Possible cause	Recommended action
Low target yield	The target protein has been degraded.	Add protease inhibitors.
	The amount of magnetic beads used is incorrect.	Test different amounts of magnetic beads used for capture.
	The sample contains a low amount of target protein.	Increase incubation time of the beads and/or antibody with the target.
		Increase incubation time with the elution buffer or use more stringent elution conditions.
Protein does not elute	Elution conditions are too mild.	Increase incubation time with elution buffer or use more stringent elution conditions.
Unwanted bands appear on Western blot	Nonspecific proteins are bound to the magnetic beads.	Add 50-350 mM of NaCl to the Binding/Wash and Elution Buffers.
Recovered protein is inactive	Elution conditions are too stringent.	Use a milder elution buffer.
Magnetic beads aggregate during immunoprecipitation procedure	Buffer is incompatible with magnetic beads or pH was changed.	Handle the beads as directed in the instructions.
		Aggregation may have no impact on functionality and could be cross binding.
Beads appear to be aggregated	Some aggregation may occur during prolonged storage of beads.	Apply short sonication of the product vial for approximately 5–10 minutes in a sonication bath.

Sustainable workflow options

Alternative more sustainable reagents may be used, for example Tween™ 20 may be replaced with biodegradable surfactants.

Recycling instructions

The bottle used to supply DynaGreen™ magnetic beads is produced from recyclable material. The 0.5 mL volume is supplied in a bottle produced from Polypropylene (PP) plastic. The 3 mL and 25 mL volumes are supplied in bottles produced from High-density polyethylene (HDPE) plastic. We recommend you dispose of remaining product in compliance with the requirements of applicable local regional or national/federal regulations prior to sending the bottle to appropriate recycling.

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0028533

Revision	Date	Description
C.0	31 May 2023	DynaGreen™ CaptureSelect™ Anti-IgG-Fc (ms) was added to the manual.
B.0	10 January 2023	Patent pending information was removed from the manual.
A.0	13 December 2022	New document for manual immunoprecipitation using DynaGreen™ Protein A and DynaGreen™ Protein A/G magnetic beads.