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

Automated protocols for direct or indirect immunoprecipitation

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

Pub. No. MAN0028534 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.

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.

Procedure overview

The principle of isolation is easy, the target-specific antibodies bind to the DynaGreen™ magnetic beads through their Fc regions during a short incubation, followed by washing and elution. All plates need to be prefilled with DynaGreen™ magnetic beads, antibody (Ab), and the required buffers before running the protocols (see “Set up processing plates” on page 4). The automated protocols are performed in only 40 minutes from start to finish. The protocols can take up to 96 samples/run for the KingFisher™ Apex Purification System and KingFisher™ Flex Purification System and 12 samples/ run for KingFisher™ Duo Prime Purification System.

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
KingFisher™ Purification Systems	
KingFisher™ Apex Purification System with 96 Deep-Well Head	A5400930
KingFisher™ Flex Purification System with 96 Deep-Well Head	5400630
KingFisher™ Duo Prime Purification System with 12 Deep-Well Head	5400110
KingFisher™ Deep-Well 96 Plate, V-bottom, poyproylene (50–1000 µL)	95040450
KingFisher™ Apex Purification System 96 Tip Comb for Deep-Well Magnets	97002570
KingFisher™ Flex Purification System 96 Tip Comb for Deep-Well Magnets	97002820
KingFisher™ Duo Prime Purification System 12 Tip Comb for Deep-Well Magnets	97003500
General materials	
1.5 mL microcentrifuge tubes	MLS
Phosphate-buffered saline (PBS), pH 7.4 [1]	10010023
Glycine (50 mM), pH 2.8	MLS
NuPAGE™ LDS Sample Buffer	NP0007
NuPAGE™ Sample Reducing Agent	NP0009
Antibody for immunoprecipitation	MLS
RIPA Lysis and Extraction Buffer	89900

[1] 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. Use ~5 µg Ab/mg beads (~2.5 µg antibody in 200 µL of PBS-T per well).
- For standard immunoprecipitation, use PBS for Ab 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.
- The automated protocol is a good starting point for immunoprecipitation or protein purification, but optimization of some parameters might be required, depending on your sample type, downstream application, or if you are working with different volumes (see “Optimize run parameters” on page 5).

Before you begin

- Resuspend DynaGreen™ bead reagents by vortexing > 30 seconds and by tilting and rotating the vial for at least 5 minutes. Visually inspect the beads to ensure they are fully resuspended before adding the beads to Plate 1.

- Combine the Tip Comb with a Deep Well 96 Plate. See the instrument user manual for detailed instructions.
- The elution volume is optimized for Western blot protocols. Increase the elution volume to obtain a higher protein yield for other applications.
- If using Bolt™ LDS Sample Buffer in a heated elution, install the KingFisher™ Flex Heating Block (see user manual for proper installation) to heat samples at 70°C for 10 minutes.
- If you select SDS-PAGE reducing sample buffer for elution and will be performing a Western blot using rabbit antibodies (primary or secondary) do not heat the samples. Incubate at room temperature for 10 minutes.
- If low-pH elution buffer is selected for elution, the eluate can be neutralized using 15 µL 1M Tris pH 7.5 for each 100 µL of eluate upon run completion.
- To limit evaporation, select Mix and Medium speed under the subheading Heating Action.

Download immunoprecipitation protocol

The following immunoprecipitation protocol is designed for use with the KingFisher™ Apex Purification System, KingFisher™ Flex Purification System, and KingFisher™ Duo Prime Purification System. The protocol can be modified according to your needs using the BindIt™ Software provided with the instrument. For detailed instructions to import protocols to KingFisher™ instruments from an external computer, see the BindIt™ Software user guide.

1. Go to **thermofisher.com/automation**
2. Navigate to instrument model.
3. In the left panel, open **BindIt™ Software** and protocols for KingFisher™ Systems.
4. Scroll down to Protocols for BindIt™ Software, then open **View all protein purification protocols** for BindIt™ Software.
5. Find your product, chose direct protocol, then click on the KingFisher™ instrument to start downloading the BindIt™ Software.

Set up processing plates

Add processing reagents to the indicated positions, according to your instrument and chosen IP protocol. For a direct IP protocol, see Table 1 and Table 2. For an indirect IP protocol, see Table 3 and Table 4.

Table 1 Set up plate for direct protocol on KingFisher™ Apex Purification System and KingFisher™ Flex Purification System

Row ID	Plate number	Reagent	Volume per well
Elution	1	Elution Buffer	27 µL
Tip Comb	2	Place Tip Comb in Row 2.	
Beads	3	Beads	0.5 mg/25 µL
Antibody	4	Antibody	200 µL
Wash I	5	PBS/Tween™ 20 0.5%	200 µL
Cell lysate	6	Cell lysate	200 µL
Wash II	7	PBS	200 µL
Wash III	8	PBS	200 µL

Table 2 Set up plate for direct protocol on KingFisher™ Duo Prime Purification System

Row ID	Plate row	Reagent	Volume per well
Elution	A	Elution Buffer	27 µL
Tip Comb	B	Place Tip Comb in Row B.	
Beads	C	Beads	0.5 mg/ 25 µL
Antibody	D	Antibody	200 µL
Wash I	E	PBS/Tween™ 20 0.5%	200 µL
Cell lysate	F	Cell lysate	200 µL
Wash II	G	PBS	200 µL
Wash III	H	PBS	200 µL

Table 3 Set up plate for indirect protocol on KingFisher™ Apex Purification System and KingFisher™ Flex Purification System

Row ID	Plate number	Reagent	Volume per well
Elution	1	Elution Buffer	27 µL
Tip Comb	2	Place Tip Comb in Row 2.	
Beads	3	Beads	0.5 mg/25 µL
Antibody + Target	4	Antibody + Target after 10 minutes incubation	200 µL
Wash I	5	PBS	200 µL
Wash III	6	PBS	200 µL

Table 4 Set up plate for indirect protocol on KingFisher™ Duo Prime Purification System

Row ID	Plate row	Reagent	Volume per well
Elution	A	Elution Buffer	27 µL
Tip Comb	B	Place Tip Comb in Row B.	
Beads	C	Beads	0.5 mg/ 25 µL
Antibody + Target	D	Antibody + Target after 10 minutes incubation	200 µL
Wash I	E	PBS	200 µL
Wash III	F	PBS	200 µL

Run the automated immunoprecipitation protocol

1. Select the appropriate protocol on the instrument, then press **Start**.
2. Open the instrument door, then load the plates into the instrument when prompted, pressing **Start** after loading each plate.
3. At the end of the run, remove the plates from the instrument when prompted, pressing **Start** after removing each plate.
4. Press **Stop**.

Optimize run parameters

If you change any of the volumes recommended in the tables for the KingFisher™ instruments, the BindIt™ protocol on the KingFisher™ instrument also must be changed. The following parameters can be optimized:

- Titrate the amount of beads per sample to optimize the conditions towards your downstream application and the sensitivity of the antibody to the antigen.
- The total antibody volume is not critical for the immunoprecipitation protocol as long as the antibody amount is consistent with the amount of DynaGreen™ bead reagent used (use ~5 ug antibody/mg of DynaGreen™ bead reagent).
- If the target protein is available in low abundance or the affinity of the antibody is low, the incubation time of the antibody-coated beads with the sample can be increased from 10 minutes to 1 hour to increase the protein yield, and/or chose the indirect technique.
- The elution volume in the protocol is based on Western blot as the downstream assay (27 µL). The elution volume can be increased depending on your downstream application.
- The protocol is set up for denaturing elution conditions using LDS sample buffer (70°C), but mild elution conditions can be used (50 mM Glycine pH 2.8).
- Depending on the starting sample and your downstream application, the buffer volume, or starting sample volume can be scaled up or down as required.
- 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 antibody 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.

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.

Frequently asked questions

Question	Answer
What plates are compatible with the KingFisher™ Apex and KingFisher™ Flex instruments?	The KingFisher™ Apex and KingFisher™ Flex instruments are compatible with KingFisher™ 24 Deep-Well Plates, KingFisher™ Deep-Well 96 Plates, KingFisher™ 96 and 96 PCR plates.
Is it possible to concentrate samples during the run?	Both deep-well plates and KingFisher™ 96 plates can be used during the same run. Therefore, it is possible to start processing using larger volumes (in a deep-well plate), then elute the purified sample to a smaller volume (in a KingFisher™ 96 plate).
Is it possible to heat samples during the run?	The heating block is located inside the instrument and can be used automatically during the sampling process. All plates compatible with the KingFisher™ Apex and KingFisher™ Flex instruments can be heated using specially designed, interchangeable heating blocks.
Why do the beads stick to the plastic tips and wells or the eluted protein sticks to the wells?	Proteins conjugate to beads and eluted proteins can non-specifically bind to plastics. Adding detergent (0.05%-0.1% Tween™ 20) to Binding/Wash or Elution Buffers prevents the protein conjugated to the beads from sticking. Alternatively, silanize the elution plate.
Are the reagent volumes in each well critical?	The protocols are optimized for the recommended volumes. For what can be successfully changed, see “Optimize run parameters” on page 5.

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.

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**.

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

Revision history: Pub. No. MAN0028534

Revision	Date	Description
C.0	6 June 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 automated immunoprecipitation using DynaGreen™ Protein A and DynaGreen™ Protein A/G magnetic beads.