invitrogen

Automated immunoprecipitation protocols for Dynabeads™ Protein A or Dynabeads™ Protein G on KingFisher ™ Flex

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The protocols for automated immunoprecipitation using the KingFisher™ Flex instrument are compatible with the following products.

Product name	Cat. no.	Dynabeads™ volume/#tests	Protocol name
Dynabeads™ Protein A	10001D 10002D 10008D	1 mL/20 5 mL/100 50 mL/1000	IP Dynabeads M-280 Protein A and G Direct[2] IP Dynabeads M-280 Protein A and G Indirect[2]
Immunoprecipitation Kit Dynabeads™ Protein A[1]	10006D	2 mL/40	
Dynabeads™ Protein G	10003D 10004D 10009D	1 mL/20 5 mL/100 50 mL/1000	
Immunoprecipitation Kit Dynabeads™ Protein G[1]	10007D	2 mL/40	

Dynabeads™ Protein A and Dynabeads™ Protein G contain 30 mg Dynabeads™/mL in phosphate buffered saline (PBS), pH 7.4, with 0.01% Tween™-20 and 0.09% sodium azide as a preservative.

[1] The immunoprecipitation kits also include binding-, washing-, and elution buffers. For detailed product description and data, or to get the manual immunoprecipitation protocol, visit the Dynabeads™ product specific page (e.g. search 10001D) on www.thermofisher.com.

[2] To request the required BindIt software or to get the script files downloaded to your computer and then transferred to the KingFisher Flex and Duo Prime instruments, visit www.thermofisher.com/automation.

Product description

Dynabeads™ Protein A and Dynabeads™ Protein G are designed for immunoprecipitation (IP) of proteins, protein complexes, protein-nucleic acid complexes, and other antigens (Ag). The principle of isolation is easy; in the direct technique a target protein specific antibody (Ab) is incubated with Dynabeads™ followed by a wash to remove unbound antibodies. The Ab-bound beads are then incubated with the protein containing sample for capture, followed by washing and elution. In the indirect technique, the Ab is first added to the sample prior to adding the beads, followed by washing and elution. Start with the direct technique. Chose the indirect technique if the target protein is of low abundance or the affinity of the Ab is low.

All plates need to be prefilled with Dynabeads™, Ab, and the required buffers before running the protocols (for details, see table 1 for direct technique and table 2 for indirect technique). The automated protocols are performed in only 40 minutes from start to finish and can take up to 96 samples/run.

Required materials

- KingFisher™ Flex with 96 Deep-Well Head
- KingFisher™ Deep-Well 96 Plate, V-bottom, polypropylene (50-1000µL)
- KingFisher™ Flex 96 Tip Comb for Deep-Well Magnets
- 1.5–mL micro centrifuge tubes
- Binding/Washing Buffer: Phosphate-buffered saline (PBS) containing 0.05% Tween-20 and 0.5M NaCl
- Elution Buffer: IgG Elution Buffer, pH 2.0 or 50mM glycine, pH 2.8
- Alternative Elution Buffer: SDS-PAGE reducing sample buffer or 4x Bolt™ LDS Sample Buffer (reducing or non-reducing conditions depends on the detection of the downstream target)
- Antibody for immunoprecipitation
- Cell Lysis Buffer (e.g. Pierce Lysis Buffer)
- Neutralization Buffer: High-ionic strength alkaline buffer, e.g. 1M phosphate or 1M Tris; pH 7.5-9

Download the immunoprecipition protocol

The following immunoprecipitation protocol is designed for use with the KingFisher™ Flex instrument. The protocol can be modified according to your needs using the BindIt Software provided with the instrument.

For detailed instructions to import protocols to the KingFisher™ Flex instruments from an external computer, see the BindIt software user guide.

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

Set up the processing plates

Set up the processing plates for direct protocol (Table 1) or for indirect protocol (Table 2).

The amount of antibody need to be titrated for each experiment. Use ~5 µg antibody/mg beads (~7–8 µg antibody in 200 µL of PBS-T per well).

Table 1. Processing plates for direct protocol

Plate position	Plate name	Reagents	Volume per well
1	Dynabeads	Dynabeads™	50 µL
2	Antibody	Antibody in PBS-T (0.02% Tween-20)	200 µL
3	Wash I	PBS-T	200 µL
4	Target	Cell lysate	200 µL
5	Wash II	PBS	200 µL
6	Wash II	PBS	200 µL
7	Elution	LDS	30 µL

Table 2. Processing plates for indirect protocol

Plate position	Plate name	Reagents	Volume per well
1	Dynabeads	Dynabeads™	50 µL
2	Antibody + Target	Antibody + Cell lysate[1]	200 µL
3	Wash II	PBS	200 µL
4	Wash II	PBS	200 µL
5	Elution	LDS	30 µL

[1] Incubate the antibody with the cell lysate for at least 10 minutes before running the protocol.

Notes:

- To ensure bead homogeneity, resuspend Dynabeads™ by vortexing > 30 seconds or by tilting and rotating the vial for 5 minutes 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 100 µL 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, neutralize the pH using 15 µL Neutralization Buffer for each 100 µL of eluate upon run completion.
- To limit evaporation, select **Mix** and **Medium** speed under the subheading **Heating Action**.

Run the automated immunoprecipitation protocol

1. Selet 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 the run parameters

The automated protocol is a good starting point for IP or protein purification, but optimization of some parameters might be required, dependent on your sample type, downstream application, or if you are working with different volumes.

Note: If you change any of the volumes recommended in Table 1 or Table 2, the BindIt protocol on the KingFisher™ instrument also need to be changed

The following parameters can be optimized:

- **Bead volume**. Titrate the amount of beads per sample to optimize the conditions towards your downstream application and vs. the sensitivity of the antibody to the antigen.
- **Antibody volume**. The total antibody volume is not critical for the immunoprecipitation protocol as long as the antibody amount is consistent with the amount of Dynabeads™ (use ~5 ug antibody/mg of Dynabeads™).
- **Dynabeads™ incubation time with the sample.** 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.
- **Elution volume**. The elution volume in the protocol is based on Western blot as the downstream assay (30 µL). By increasing the elution volume to 100 µL more protein is eluted off.
- **Elution condition**s. The protocol is set up for denaturing elution conditions using LDS sample buffer (70°C), but you can change it to mild elution conditions.
- **Sample or buffer volume.** Dependent on your starting sample and your downstream application, the buffer volume, or starting sample volume can be scaled up or down as required.

Troubleshooting

Observation	Possible cause	Recommended actions
Low amount of protein was recovered	The protein degraded	Add protease inhitors
	Not enough magnetic beads were used	Increase the amount of magnetic beads used for capture
	Sample had an insufficient amount of target protein	Increase incubation time with the elution buffer or use more stringent elution conditions
Protein does not elute	Elution conditions were too mild	Increase incubation time with elution buffer or use more stringent elution conditions
Bands at ~50kDa appear on the Western blot	Nonspecific protein bound to the magnetic beads	Add 50-350 mM of NaCl to the Binding/Wash and Elution Buffers
Recovered protein was inactive	Elution conditions were too stringent	Use a milder elution buffer
Magnetic beads aggregated	Magnetic beads were frozen or centrifuged	Handle the beads as directed in the instructions
	Buffer was incompatible with magnetic beads or pH was changed	Handle the beads as directed in the instructions

Frequently asked questions about the KingFisher™ instrument

Question	Answer
Which plates are compatible with KingFisher™ Flex Instrument?	The KingFisher™ Flex Instrument is compatible with the 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. It is possible to start the processing using larger volumes (Deep-Well Plate) and elute the purified sample in a smaller volume (KingFisher 96 Plate).
Is it possible to heat the samples during the run?	The heating block is located inside the instrument and can be used automatically during the sample process. All plates compatible with the KingFisher™ Flex Instrument 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, but see section "Optimize the run parameters" for what can be successfully changed.

Description of materials

The products contain Dynabeads™ Protein A or Dynabeads™ Protein G for immunoprecipitation. The Dynabeads™ are uniform, 2.8 µm, superparamagnetic beads with either recombinant protein A (approximately 45 kDa) or recombinant protein G (approximately 17 kDa) covalently coupled to the surface. See the product specific pages on www.thermofisher.com for details of the buffers in the kit version.

Related products

Product	Cat. no.
KingFisher™ instruments and accessories	
KingFisher™ Flex Magnetic Particle Processor with 96	5400630
KingFisher™ Flex Magnetic Particle Processor with 24	5400640
KingFisher™ Deep-Well 96 Plate	95040450
KingFisher™ Flex 96 Tip Comb for Deep-Well Magnets	97002534
Dynabeads™ for automated immunoprecipitation	
Dynabeads™ Sheep anti-Mouse IgG	11202D
Dynabeads™ Sheep anti-Rabbit IgG	11204D
Dynabeads™ M-280 Streptavidin	11206D
Buffers and reagents	
NP40 Cell Lysis buffer	FNN0021
Pierce 20X TBS Tween™ 20 Buffer	28360
Pierce IgG Elution Buffer, pH 2.0	21028
4X Bolt LDS Sample Buffer	B0007
SeeBlue Plus2 Pre-stained Protein Standard	LC5925
20x Bolt MES SDS Running Buffer	B000202
Bolt 4-12% Bis-Tris Plus Gels, 10-well	NW04120BOX
Bolt Transfer Buffer (20X)	BT00061
SuperSignal West Dura Extended Duration Substrate	34076

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