INSTRUCTIONS

Pierce™ Classic Magnetic IP/Co-IP Kit

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88804

Number	Description
88804	**Pierce Classic Magnetic IP/Co-IP Kit,** contains sufficient reagents to perform 40 reactions using 25µL of magnetic beads

Kit Contents:

Pierce Protein A/G Magnetic Beads, 1mL supplied at 10mg/mL in water containing 0.05% NaN_3

Pierce IP Lysis/Wash Buffer, 2 × 50mL, pH 7.4, 0.025M Tris, 0.15M NaCl, 0.001M EDTA, 1% NP40, 5% glycerol

Lane Marker Sample Buffer, Non-reducing, (5X), 5mL, pH 6.8, 0.3M Tris·HCl, 5% SDS, 50% glycerol, lane marker tracking dye

Elution Buffer, 5mL, pH 2.0

Neutralization Buffer, 0.5mL, pH 8.5

Storage: Upon receipt store at 4°C. Product is shipped with an ice pack.

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Introduction

The Thermo Scientific™ Pierce™ Classic Magnetic IP/Co-IP Kit enables highly effective and efficient antigen immunoprecipitation (IP) and co-immunoprecipitation (co-IP) using less than 10µg of antibody without centrifugation. The specific antibody is first added to the sample to form an immune complex that is then bound to the magnetic beads. The complex is washed to remove non-bound material and a low-pH elution buffer dissociates the bound immune complex from the Protein A/G. Alternatively, the Lane Marker Sample Buffer is included for dissociation using denaturing conditions or for downstream sample prep for SDS-PAGE analysis. The kit includes Thermo Scientific™ Pierce™ Protein A/G Magnetic Beads for fast and convenient magnetic isolation of antigens and optimized buffers for high antigen yield. The beads are removed from the solution manually using a magnetic stand or by automation with an instrument such as the Thermo Scientific KingFisher Flex Instrument.

The recombinant Protein A/G (~50.5kDa; apparent molecular weight by SDS-PAGE ~40-45K) immobilized onto the magnetic beads combines the IgG binding domains of both Protein A and Protein G. Protein A/G contains four Fc-binding domains from Protein A and two from Protein G, making it a convenient tool for investigating and purifying immunoglobulins.

Procedure Summary

1. Incubate cell lysate with chosen antibody for IP for 1-2 hours at room temperature (RT) or overnight at 4ºC.
2. Bind antigen/antibody complex to Protein A/G magnetic beads for 1 hour at RT.
3. Wash beads twice with IP Lysis/Wash Buffer and once with purified water.
4. Elute the antigen/antibody complex.

Important Product Information

- Do not centrifuge, dry or freeze the magnetic beads, as this can cause the beads to aggregate and lose binding activity.
- Co-elution of antibody with the immunoprecipitated antigen occurs with this kit. Consequently, there could be at least three protein bands on a reducing SDS-PAGE gel or Western blot; the antibody heavy chain (50kDa), the antibody light chain (25kDa) and the antigen. If an antibody masks the IP antigen, use the Thermo Scientific Clean-Blot IP Detection Reagent (Product No. 21230 and 21233).
- For optimal results, use an affinity-purified antibody. Although serum may be used, the antibody that is specific for the antigen of interest may comprise only 1-2% of the total IgG in the serum sample and will result in low antigen yields.
- IP Lysis/Wash Buffer has been tested on representative cell types including, but not limited to, the following cell lines: HeLa, Jurkat, A431, A549, MOPC, NIH 3T3 and U2OS. Typically, 10^6 HeLa cells yield ~10mg of cell pellet and ~3µg/µL (or 300µg) when lysed with 100µL of IP Lysis/Wash Buffer.
- To minimize protein degradation, include protease inhibitors (e.g., Thermo Scientific Halt Protease Inhibitor Single-Use Cocktail EDTA-free, Product No. 78425) in preparation of cell lysates.
- The IP Lysis/Wash Buffer is compatible with the Thermo Scientific Pierce BCA Protein Assay (Product No. 23225).
- A low-pH elution can be used for single-use applications. Optimal time for low-pH elution is 10 minutes; exceeding 10 minutes may result in yield reduction.
- When using rabbit antibodies (primary or secondary) in downstream Western blot applications and performing elution in Lane Marker Sample Buffer, do not heat the beads. For all other antibody species, boiling the beads in Lane Marker Sample Buffer is acceptable for single-use applications. If boiled, beads should not be reused as boiling may cause bead aggregation and loss of binding activity.

Additional Materials Required

- Phosphate-buffered saline (PBS, 100mM sodium phosphate, 100mM NaCl; pH 7.2; Product No. 28372)
- Dithiothreitol (DTT; Product No. 20290 or 20291); optional (for reducing elution only)
- Antibody for IP
- Antigen Sample

For Automated IP:

- Thermo Scientific™ KingFisher™ Flex System with 96 Deep Well Head (Product No. 5400630) or
- Thermo Scientific™ Microtiter Deep Well 96 Plate, V-bottom, polypropylene (100-1000µL; Product No. 95040450)
- KingFisher Flex 96 Tip Comb for Deep Well Magnets (Product No. 97002534)

For Manual IP:

- Magnetic stand (e.g., DynaMag™ 2 Magnet, Product No. 12321D))

Procedure for the Pierce Classic Magnetic IP Kit

Mammalian Cell Lysis

Protocol I: Lysis of Cell Monolayer (Adherent) Cultures

1. Carefully remove culture medium from confluent cells.
2. Wash the cells once with PBS.
3. Add ice-cold IP Lysis/Wash Buffer (Table 1) to the cells. Incubate on ice for 5 minutes with periodic mixing.

Table 1. Suggested volume of IP Lysis/Wash Buffer to use for different standard culture plates.

Plate Size/Surface Area	Volume of IP Lysis/Wash Buffer
100 × 100mm	500-1000µL
100 × 60mm	250-500µL
6-well plate	200-400µL per well
24-well plate	100-200µL per well

4. Transfer the lysate to a microcentrifuge tube and centrifuge at ~13,000 × *g* for 10 minutes to pellet the cell debris.
5. Transfer supernatant to a new tube for protein concentration determination and further analysis.

Protocol II: Lysis of Cell Suspension Cultures

1. Centrifuge the cell suspension at 1000 × *g* for 5 minutes to pellet the cells. Discard the supernatant.
2. Wash cells once by suspending the cell pellet in PBS. Centrifuge at 1000 × *g* for 5 minutes to pellet cells.
3. Add ice cold IP Lysis/Wash Buffer to the cell pellet. Use 500µL of IP Lysis/Wash Buffer per 50mg of wet cell pellet (i.e., 10:1 v/w). If using a large amount of cells, first add 10% of the final volume of IP Lysis/Wash Buffer to the pellet and pipette the mixture up and down to mix. Add the remaining volume IP Lysis/Wash Buffer to the cell suspension.
4. Incubate lysate on ice for 5 minutes with periodic mixing. Remove cell debris by centrifugation at ~13,000 × *g* for 10 minutes.
5. Transfer supernatant to a new tube for protein concentration determination and further analysis.

A. Preparation of the Immune Complex

Note: The amount of sample needed and the incubation time are dependent upon each specific antibody-antigen system and may require optimization for maximum yield. The following protocol is for 2-10µg of affinity-purified antibody and can be scaled up as needed.

1. Combine cell lysate with 2-10µg of IP antibody per sample in a microcentrifuge tube. The suggested amount of total protein per IP reaction is 500-1000µg, as determined by the Pierce BCA Protein Assay.
2. Dilute the antibody/lysate solution to 500µL with IP Lysis/Wash Buffer.
3. Incubate for 1-2 hours at RT or overnight at 4ºC to form the immune complex.

B. Manual Immunoprecipitation

Note: To ensure bead homogeneity, mix the vial thoroughly by repeated inversion, gentle vortexing or using a rotating platform.

1. Place 25µL (0.25mg) of Pierce Protein A/G Magnetic Beads into a 1.5mL microcentrifuge tube.
2. Add 175µL of IP Lysis/Wash Buffer to the beads and gently vortex to mix.
3. Place the tube into a magnetic stand to collect the beads against the side of the tube. Remove and discard the supernatant.
4. Add 1mL of IP Lysis/Wash Buffer to the tube. Invert the tube several times or gently vortex to mix for 1 minute. Collect beads with magnetic stand. Remove and discard the supernatant.

5. Add the antigen sample/antibody mixture (Section B) to the tube containing pre-washed magnetic beads and incubate at room temperature for 1 hour with mixing.
6. Collect the beads with a magnetic stand, remove the unbound sample and save for analysis.
7. Add 500µL of IP Lysis/Wash Buffer to the tube and gently mix. Collect the beads and discard the supernatant. Repeat this wash twice.
8. Add 500µL of ultra pure water to the tube and gently mix. Collect the beads on a magnetic stand and discard the supernatant.
9. Low-pH Elution: Add 100µL of Elution Buffer to the tube. Incubate the tube at RT with mixing for 10 minutes. Magnetically separate the beads and save the supernatant containing the target antigen. To neutralize the low pH, add 10µL of Neutralization Buffer for each 100µL of eluate.

   Alternative Elution: Add 100µL of Lane Marker Sample Buffer (diluted five-fold with purified water) to the tube and heat the samples at 96-100ºC in a heating block for 10 minutes. Magnetically separate the beads and save the supernatant-containing target antigen.

   Note: If you will be performing a Western blot using rabbit antibodies (primary or secondary), do not heat the samples. Incubate at RT for 10 minutes with mixing.

   Note: If elution under reducing conditions is desired, add DTT (to a final concentration of 50mM) to the 1X Lane Marker Sample Buffer.

C. Automated Immunoprecipitation

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

1. Download the "Classic Immunoprecipitation" protocol from the Thermo Fisher Scientific website (*thermofisher.com/binditprotocols*) into the BindIt Software on an external computer.
2. Transfer the protocol to the KingFisher Flex from an external computer. See the BindIt Software User Manual for detailed instructions on importing protocols.
3. Set up plates according to Table 2.

Table 2. Pipetting instructions for the IP protocol using the Microtiter Deep Well 96 Plates.

Plate #	Plate Name	Content	Volume	Time/Speed
1	Beads	Protein A/G Beads	25µL	5 seconds
		IP Lysis/Wash Buffer	175µL	
2	Bead Wash	IP Lysis/Wash Buffer	1000µL	1 minute/Slow
3	Bind	Antibody/Antigen Sample	500µL	1 hour/Slow
4	Wash 1	IP Lysis/Wash Buffer	500µL	30 seconds/Slow
5	Wash 2	IP Lysis/Wash Buffer	500µL	30 seconds/Slow
6	Wash 3	Ultrapure Water	500µL	30 seconds/Slow
7	Low pH Elution	Elution Buffer	100µL	10 minutes/Medium
	Denaturing Elution	Lane Marker Sample Buffer		
8	Tip Plate	KingFisher Flex 96 Tip Comb for Deep Well Magnets	-	10 seconds/Fast

4. Select the protocol using the arrow keys on the instrument keypad and press Start. See the KingFisher Flex Instrument User Manual for detailed information.
5. Slide open the door of the instrument's protective cover.
6. Load plates into the instrument according to the protocol request, placing each plate in the same orientation. Confirm each action by pressing Start.
7. After the samples are processed, remove the plates as instructed by the instrument's display. Press Start after removing each plate. Press Stop after all the plates are removed.

Notes:

- If fewer than 96 wells are used, fill the same wells in each plate. For example, if using wells A1 through A12, use these same wells in all plates.
- To ensure bead homogeneity, mix the vial thoroughly by repeated inversion, gentle vortexing, or rotating platform 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 beads can be eluted into 100µL of 0.1M glycine, pH 2.0 or 100µL of SDS-PAGE reducing sample buffer.
- If using SDS-PAGE reducing sample buffer in a heated elution, install the KingFisher Flex Heating Block (see manual for proper installation) to heat samples at 96-100ºC for 10 minutes.

  Note: 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 is selected for elution, neutralize the pH using 10µL Neutralization Buffer for each 100µL of eluate upon run completion.
- To limit evaporation, select "Mix" and "Slow" speed under the subheading "Heating Action."
- If reducing agent is desired, add 50mM DTT to 1X Lane Marker Sample Buffer.

Troubleshooting

Problem	Possible Cause	Solution
Antigen did not immunoprecipitate	Sample did not contain sufficient antigen to detect	Verify protein expression and/or lysis efficiency of the lysate by SDS-PAGE or Western blot; add more sample if required
	Antibody could not bind antigen	Use a recent source of the specific antibody or an alternative antibody that recognizes a different epitope
	Component in the IP Lysis/Wash Buffer interfered with antibody-antigen binding	Perform the IP and washes using alternate buffer (e.g., 0.5% CHAPS in TBS)
Eluted antibody bands mask antigen of interest	Antigen had a molecular weight of approximately 50kDa or 25kDa	Use Thermo Scientific™ Clean-Blot™ IP Detection Reagents (Product No. 21230 or 21233) for Western blot detection
		Use a different antibody species for Western blot from the antibody species used for IP (i.e., IP with mouse IgG and detect with rabbit IgG)
		Use Pierce Direct IP Kit (Product No. 26148) or Pierce Crosslink IP Kit (Product No. 26147) to immobilize antibody to the resin
		Do not reduce samples before SDS-PAGE so the antibody migrates at 150kDa
Low amount of recovered protein	The protein degraded	Add protease inhibitors
	Not enough magnetic beads were used	Increase the amount of magnetic beads used for capture
	Sample had an insufficient amount of target protein	Increase amount of antigen sample
Protein does not elute	Elution conditions were too mild	Increase incubation time with elution buffer to 15 minutes or use more stringent elution buffer
Bands at ~50kDa appeared on the Western blot	Elution was performed in Lane Marker Sample Buffer at temperatures greater than RT in conjunction with a rabbit antibody being used for Western blot detection	Perform elution at room temperature when using rabbit antibodies

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Multiple nonspecific bands	Nonspecific protein bound to the magnetic beads	Add 50-350mM NaCl to the Binding/Wash and Elution Buffers or pre-clear sample by incubating with Pierce Protein A/G Magnetic Beads without antibody before forming the immune complex
Recovered protein was inactive	Elution conditions were too stringent	Use a milder elution buffer (e.g., Pierce Gentle Ag/Ab Elution Buffer, Product No. 21027)
Magnetic beads aggregated	Magnetic beads were frozen or centrifuged	Handle the beads as directed in the instructions
	Buffer was incompatible with magnetic beads	

Additional Information Available on Our Website

- Frequently Asked Questions
- Tech Tip #43: Protein Stability and Storage
- Tech Tip #34: Binding characteristics for immunoglobulins and Protein A, G, A/G and L
- Visit thermofisher.com/kingfisher for information on KingFisher Products

Frequently Asked Questions for the Thermo Scientific KingFisher Instrument

Question	Answer
Which plates are compatible with KingFisher Flex Instruments?	The KingFisher Flex is compatible with the KingFisher 24 Deep Well Plates, Microtiter 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 the processing using larger volumes (in a deep well plate) and elute the purified sample to a smaller volume (in a 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.
Are the reagent volumes in each well critical?	For best results, keep the specified volumes within defined limits to avoid spillover.

Related Products

88802-3 **Pierce Protein A/G Magnetic Beads**
88805 **Pierce Crosslink Magnetic IP/Co-IP Kit**
88845-6 **Pierce Protein A Magnetic Beads**
88847-8 **Pierce Protein G Magnetic Beads**
88849-50 **Pierce Protein L Magnetic Beads**
88826-7 **Pierce NHS-Activated Magnetic Beads**
88828 **Pierce Direct Magnetic IP/Co-IP Kit**
88816-7 **Pierce Streptavidin Magnetic Beads**
24615 **Imperial™ Protein Stain**
34075 **SuperSignal™ West Dura Extended Duration Substrate**
XP04200BOX **Novex™ Tris-Glycine protein gels** (see thermofisher.com/proteingels for a complete listing)
78440 **Halt™ Protease and Phosphatase Inhibitor Cocktail (100X)**
78430 **Halt Protease Inhibitor Single-Use Cocktail (100X)**

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Current product instructions are available at thermofisher.com. For a faxed copy, call 800-874-3723 or contact your local distributor.

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