INSTRUCTIONS

Pierce MS-Compatible Magnetic IP Kit (Protein A/G)

90409 2589.1

Number	Description
90409	**Pierce MS-Compatible Magnetic IP Kit (Protein A/G),** 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
	IP-MS Cell Lysis Buffer, 100mL
	IP-MS Wash Buffer A, 75mL
	IP-MS Wash Buffer B, 40mL
	$MgCl_2$ (1M), 0.75mL
	IP-MS Elution Buffer, 6mL
	Storage: Upon receipt store at 4°C. Product is shipped with an ice pack.

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Introduction

The Thermo Scientific™ Pierce™ MS-Compatible Magnetic IP Kit (Protein A/G) enables highly effective antigen immunoprecipitation (IP) and co-immunoprecipitation (co-IP) for mass spectrometry LC-MS analysis. The high capacity Protein A/G magnetic beads maximize the recovery of low-abundant targets while their low non-specific binding minimizes background protein identifications. Cells are first lysed in a non-ionic detergent-containing buffer and incubated with a specific antibody to form an immune complex. The bound complex is washed thoroughly with detergent-free buffers to greatly reduce non-specific binding and to remove any residual detergent. A low-pH elution buffer dissociates the bound immune complex from the Protein A/G, which can go directly into an in-solution digestion method followed by MS analysis; no SDS-PAGE purification is required.

IP is commonly used upstream of MS as an enrichment tool for low-abundant protein targets from complex samples, such as cell/tissue lysates or serum. However, detergents or high salt concentrations in the wash and elution buffers of classical IP procedures are incompatible with downstream MS analysis. This classical method requires upstream SDS-PAGE purification and excising bands to remove interfering salts and detergents before in-gel protein digestion. The Pierce MS-Compatible Magnetic IP Kit (Protein A/G) enables the researcher to proceed directly to in-solution digestion, eliminating the SDS-PAGE steps, saving time, minimizing sample loss, and increasing sample throughput.

Procedure Summary

1. Prepare cell lysate.
2. Incubate cell lysate with specific antibody overnight at 4ºC.
3. Bind antigen/antibody complex to Protein A/G magnetic beads for 1 hour at room temperature (RT).
4. Wash beads 3 × 500µL with IP-MS Wash Buffer A.
5. Wash beads 2 × 500µL with IP-MS Wash Buffer B.
6. Elute with 100µL of IP-MS Elution Buffer for 10 minutes at RT.
7. Dry elution in vacuum concentrator and proceed to in-solution digestion followed by MS analysis.

Important Product Information

- Do not centrifuge, dry or freeze the magnetic beads, because this can cause the beads to aggregate and lose binding activity.
- Co-elution of antibody with the immunoprecipitated antigen occurs with this kit. MS will identify some heavy and light chain peptides, however, the target identification will not be affected. If analyzed by SDS-PAGE or Western blot, there could be at least three protein bands; the antibody heavy chain (50kDa), the antibody light chain (25kDa) and the antigen.
- 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.
- For better recovery of targets using mouse IgG_1 antibody, prepare and use modified IP-MS Wash Buffer A (dilute 1M $MgCl_2$ 1:100 with IP-MS Wash Buffer A). For all other antibody subtypes, use IP-MS Wash Buffer A.
- IP-MS Cell Lysis Buffer has been tested on representative cell types including, but not limited to: HeLa, Jurkat, A431, A549, MOPC, NIH 3T3, HEK 293, HCT116, and U2OS. Typically, 10^6 HeLa cells yield ~10mg of cell pellet and ~3µg/µL (or 300µg) protein when lysed with 100µL of buffer.
- Pierce MS-Compatible Magnetic IP Kit (Protein A/G) is not compatible with samples containing IgG such as plasma/serum/CSF.
- For direct MS analysis of cell lysates, use the Thermo Scientific™ Pierce™ Mass Spec Sample Prep Kit for Cultured Cells (Product No. 84840). Sample prepared in IP-MS Cell Lysis Buffer is not directly compatible with the MS analysis.
- 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-MS Cell Lysis Buffer is compatible with the Thermo Scientific™ BCA Protein Assay (Product No. 23225), Thermo Scientific™ Pierce™ Detergent Compatible Bradford Protein Assay Kit (Product No. 23246) and Thermo Scientific™ 660 nm Protein Assay Kit (Product No. 22662).

Additional Materials Required

Note: See the "Procedure for MS Sample Preparation" section for additional materials required for that protocol.

- Phosphate-buffered saline (PBS, 100mM sodium phosphate, 100mM NaCl; pH 7.2; Product No. 28372)
- Antibody for IP
- Antigen sample
- Vacuum concentrator (e.g., Thermo Scientific™ SpeedVac™ Vacuum Concentrator)

For Manual IP:

- Magnetic stand (e.g., Thermo Scientific™ DynaMag™-2 Magnet, Product No.12321D)
- Low protein-binding microcentrifuge tubes (Product No. 90410 or 90411)

For Automated IP:

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

Procedure for the Pierce MS-Compatible 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-MS Cell Lysis Buffer (Table 1) to the cells. Incubate on ice for 10 minutes with periodic mixing.

Table 1. Suggested volume of IP-MS Cell Lysis Buffer to use for different standard culture plates.

Plate Size/Surface Area	Volume of IP-MS Cell Lysis 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 1,000 × *g* for 5 minutes to pellet the cells. Discard the supernatant.
2. Wash cells once by suspending the cell pellet in PBS. Centrifuge at 1,000 × *g* for 5 minutes to pellet cells.
3. Add ice cold IP-MS Cell Lysis Buffer to the cell pellet. Use 500µL of IP-MS Cell Lysis 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-MS Cell Lysis Buffer to the pellet and pipette the mixture up and down to mix. Add the remaining volume IP-MS Cell Lysis Buffer to the cell suspension.
4. Incubate lysate on ice for 10 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.

Immunoprecipitation

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 5µg of affinity-purified antibody and can be scaled up as needed.

1. Combine cell lysate with 5µg of IP antibody per sample in a microcentrifuge tube. The suggested amount of total protein per IP reaction is 500-1000µg.
2. Dilute the antibody/lysate solution to 500µL with IP-MS Cell Lysis Buffer.
3. Incubate overnight at 4ºC with mixing 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.

Note: For mouse IgG_1 antibody, prepare modified IP-MS Wash Buffer A by diluting 1M $MgCl_2$ 1:100 with the IP-MS Wash Buffer A.

1. Place 25µL (0.25mg) of Pierce Protein A/G Magnetic Beads into a 1.5mL low protein binding microcentrifuge tube.
2. Add 175µL of IP-MS Cell Lysis Buffer to the beads and gently vortex to mix. Place the tube into a magnetic stand to collect the beads against the side of the tube. Remove and discard the supernatant. Repeat this step once.
3. Add the antigen sample/antibody mixture (Section A) to the tube containing pre-washed magnetic beads and incubate at room temperature for 1 hour with mixing.
4. Collect the beads with a magnetic stand, remove the unbound sample and save for optional analysis by SDS-PAGE or Western blot.
5. For all antibody subtypes except mouse IgG_1, add 500µL of IP-MS Wash Buffer A to the tube and gently mix. Collect the beads and carefully remove the supernatant. Perform this wash step a total of three times.

   For mouse IgG_1 antibody, add 500µL of modified IP-MS Wash Buffer A (dilute 1M $MgCl_2$ 1:100 with the IP-MS Wash Buffer A). Collect the beads and carefully remove the supernatant. Perform this wash step a total of three times.
6. Add 500µL of IP-MS Wash Buffer B to the tube and gently mix. Collect the beads and carefully remove the supernatant. Perform this wash step a total of two times.
7. Add 100µL of IP-MS Elution Buffer to the tube. Vortex gently and incubate the tube at RT for 10 minutes. Collect the beads and transfer the supernatant containing the target antigen to a new 1.5mL low protein-binding microcentrifuge tube.
8. Dry the elution in a speed vacuum concentrator. The sample can then be processed for MS analysis (See "Procedure for MS Sample Preparation") and/or reconstituted in sample buffer to analyze by SDS-PAGE or Western blot.

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.

Note: For mouse IgG_1 antibody, prepare modified IP-MS Wash Buffer A by diluting 1M $MgCl_2$ 1:100 with the IP-MS Wash Buffer A.

1. To accommodate the number of plates and the temperature, the antibody/antigen complex must be formed before using the KingFisher Flex Instrument. Washed beads can be added to each sample in the "IP Sample" plate.
2. Download the "MS Compatible IP" protocol from the Thermo Fisher Scientific website (http://*www.thermoscientific.com/bindit-protocols*) into the BindIt Software on an external computer.
3. Transfer the protocol to the KingFisher Flex Instrument from an external computer. See the BindIt Software User Manual for detailed instructions on importing protocols.
4. 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	IP Sample	Antibody/Antigen/Bead Sample	500µL	1 hour/Slow
2	Wash 1	IP-MS Wash Buffer A (For mouse IgG1 antibody, use modified IP-MS Wash Buffer A)	500µL	15 seconds/Medium
3	Wash 2		500µL	15 seconds/Medium
4	Wash 3		500µL	15 seconds/Medium
5	Wash 4	IP-MS Wash Buffer B	500µL	15 seconds/Medium
6	Wash 5		500µL	15 seconds/Medium
7	Elution	IP-MS Elution Buffer	100µL	10 minutes/Slow
8	Tip Plate	KingFisher Flex 96 Tip Comb for Deep Well Magnets	—	10 seconds/Fast

5. Select the protocol using the arrow keys on the instrument keypad and press Start. See the KingFisher Flex Instrument User Manual for detailed information.
6. Slide open the door of the instrument's protective cover.
7. Load plates into the instrument according to the protocol request, placing each plate in the same orientation. Confirm each action by pressing Start.
8. 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.

Procedure for MS Sample Preparation

A. Additional Materials Required

- Low protein-binding microcentrifuge tubes (Product No. 90410 or 90411)
- 1M Triethylammonium bicarbonate (TEAB), 50mL (Product No. 90114)
- Urea, sequencing grade (Product No. 29700)
- Thermo Scientific™ Bond-Breaker™ TCEP Solution, Neutral pH (Product No. 77720)
- Iodoacetamide, Single-Use (Product No. 90034)
- Water, LC-MS Grade (Product No. 51140)
- Thermo Scientific™ Pierce™ Trypsin Protease, MS Grade (Product No. 90057)
- Acetic acid (e.g., Fisher Scientific Product No. A35-500)
- Trifluoroacetic acid (TFA), sequencing grade (Product No. 28904)
- Vacuum concentrator (e.g., Thermo Scientific™ SpeedVac™ Vacuum Concentrator)
- Thermomixer, heat block or incubator

B. Material Preparation

Note: Prepare all new materials before use. The amounts listed below are sufficient for preparing 24 samples.

50mM TEAB, pH 8.5	Dilute 0.5mL of 1M TEAB, pH 8.5 with 9.5mL of MS-grade water.
6M Urea/50mM TEAB, pH 8.5	Add 0.36g urea (sequencing grade) in 0.5mL of 50mM TEAB, pH 8.5, to dissolve, then bring volume to 1mL with 50mM TEAB, pH 8.5. Do not warm to dissolve.
10mM TCEP solution	Dilute 10µL 0.5M TCEP with 490µL 50mM TEAB, pH 8.5.
0.5M Iodoacetamide (IAA)	Dissolve 9.3mg of iodoacetamide in 100µL of MS-grade water. Prepare new solution and cover with foil to protect from light.
0.1µg/µL Trypsin stock solution	Dissolve 20µg of Pierce Trypsin Protease (MS grade) in 200µL of 50mM acetic acid. Store 30µL aliquots at -80°C.

C. In-Solution Sample Reduction, Alkylation and Enzymatic Protein Digestion

Note: This in-solution digestion procedure is optimized for MS-compatible IP eluates.

1. Suspend dried samples in 10µL of 6M Urea/50mM TEAB, pH 8.5 and vortex to mix. Discard any unused urea solution.
2. Add 10µL of 10mM TCEP to each sample (final TCEP concentration is 5mM). Mix and incubate at 37°C for 30 minutes in a thermomixer at 1000 rpm. If a thermomixer is unavailable, incubate the sample in a heat block or incubator. Discard any unused TCEP solution.
3. Add 1µL of IAA solution to the sample (final IAA concentration is ~24mM). Mix and incubate at room temperature for 30 minutes protected from light. Discard any unused IAA solution.
4. After alkylation with IAA, immediately add 45µL of 50mM TEAB, pH 8.5 to each sample to bring urea concentration to < 1M.
5. Prepare (just before use) 10ng/µL trypsin working solution by adding 30µL of 0.1µg/µL trypsin stock solution to 270µL of 50mM TEAB, pH 8.5.
6. Add 10µL of 10ng/µL trypsin working solution to each sample. Briefly vortex and digest overnight (16-18 hours) at 37°C in a thermomixer at 500 rpm. If a thermomixer is unavailable, incubate the sample in a heat block or incubator. Centrifuge briefly to collect condensate to bottom of tube.
7. Acidify samples by adding 2.5µL of 10% TFA (pH < 3) and vortex briefly.
8. Centrifuge at 15,000 × *g* for 2 minutes to pellet any insoluble material.
9. Transfer supernatant to a new microcentrifuge tube and store at -20°C or proceed to Step 10.
10. Clean-up digested samples with C18 spin tips (Product No. 87784) or C18 trap column (on-line/off-line) before MS analysis.

Troubleshooting

Problem	Possible Cause	Solution
Antigen did not immunoprecipitate or low amount of recovered protein	Sample did not contain sufficient antigen to detect	Verify protein expression and/or lysis efficiency by SDS-PAGE or Western blot. Use more lysate for IP if needed
	Antibody could not bind antigen or low-affinity antibody was used	Use a recent source of the specific antibody or an alternative IP-validated antibody that recognizes a different epitope
	Protein degraded	Add protease inhibitors
	Insufficient magnetic beads used	Ensure magnetic beads are evenly suspended before use
	Short liquid-chromatography (LC) gradient and slow-scanning mass spectrometer were used	Use nano LC with longer gradient (60-90 minutes) and fast-scanning mass spectrometer (e.g., Thermo Scientific™ Q Exactive™/Orbitrap™ Fusion Mass Spectrometer)
	For Mouse IgG_1 antibody, IP-MS Wash Buffer A was used instead of Modified IP-MS Wash Buffer A	Prepare and use Modified IP-MS Wash Buffer A (Dilute 1M $MgCl_2$ 1:100 with IP-MS Wash Buffer A)
	Low recovery of peptides after in-solution digestion	Use low protein-binding microcentrifuge tubes for maximum recovery of target peptides (Product No. 90410 or 90411)
Magnetic beads aggregated	Magnetic beads were frozen or centrifuged	Handle beads as directed in the instructions

Additional Information Available on Our Website

- Frequently Asked Questions
- Visit *www.thermoscientific.com/kingfisher* for information on KingFisher Products
- In the U.S.A., purchase KingFisher Supplies from Fisher Scientific. Contact your local Thermo Fisher Scientific office to purchase KingFisher Supplies outside of the U.S.A.

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.
Are the reagent volumes in each well critical?	For best results, keep the specified volumes within defined limits to avoid spillover.

Related Thermo Scientific Products

90410 **Pierce Low Protein Binding Microcentrifuge Tubes, 1.5mL**

88803 **Pierce Protein A/G Magnetic Beads, 5**mL

84840 **Pierce Mass Spec Sample Prep Kit for Cultured Cells**

90407 **Pierce™ Antibody Biotinylation Kit for IP**

90408 **Pierce™ MS-Compatible Magnetic IP Kit (Streptavidin)**

90057 **Pierce Trypsin Protease, MS Grade**

87784 **Pierce™ C18 Tips, 100µL bed**

88328 **Pierce™ HeLa Protein Digest Standard**