INSTRUCTIONS

Pierce Anti-c-Myc Magnetic Beads

88842 88843 2546.0

Number	Description
88842	**Pierce Anti-c-Myc Magnetic Beads**, 1mL, supplied at 10mg/mL in PBS containing 0.05% Tween™-20 Detergent and 0.05% NaN_3
88843	**Pierce Anti-c-Myc Magnetic Beads**, 5mL, supplied at 10mg/mL in PBS containing 0.05% Tween-20 Detergent and 0.05% NaN_3

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

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Introduction

The Thermo Scientific™ Pierce™ Anti-c-Myc Magnetic Beads are used for immunoprecipitation (IP) of specific c-Myc-tagged proteins expressed in human *in vitro* expression systems and bacterial and mammalian cell lysates. The anti-c-Myc antibody coupled to the resin is a high-affinity mouse IgG_1 monoclonal antibody that recognizes the c-Myc epitope tag (E E Q K L I S E E D L L R K R R E Q L K H K L E Q L R N S C A) derived from the human c-Myc protein. For IP, the beads are added to a sample containing c-Myc-tagged proteins. The bound c-Myc-tagged proteins are dissociated from the beads using an elution buffer. The beads are removed from the solution manually using a magnetic stand or by automation using an instrument such as the Thermo Scientific™ KingFisher™ Flex or KingFisher Duo Instrument. Automated instruments are especially useful for large-scale screening of multiple samples.

Table 1. Characteristics of the Thermo Scientific Pierce Anti-c-Myc Magnetic Beads.

Composition:	High-affinity mouse IgG_1 monoclonal antibody covalently coupled to a blocked magnetic bead surface
Magnetization:	Superparamagnetic (no magnetic memory)
Mean Diameter:	1µm (nominal)
Density:	$2.0 g/cm^3$
Bead Concentration:	10mg/mL
Binding Capacity:	≥ 10µg GST-c-Myc (26kDa fusion protein)/mg of beads *or* ≥ 10µg GST-c-Myc (26kDa fusion protein)/100µL of bead suspension

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Important Product Information

- Do not centrifuge, dry or freeze Pierce Anti-c-Myc Magnetic Beads, as this can cause the beads to aggregate and lose binding activity. To ensure good dispersal of beads for optimal antibody binding, it is important to include 0.025% to 0.1% non-ionic (e.g., Tween-20 Detergent) or zwitterionic (e.g., CHAPS) detergent in the binding and wash buffers and to mix the beads during incubation.
- For best results, determine optimal conditions for expression of c-Myc-tagged fusion protein before attempting immunoprecipitation.
- To minimize protein degradation, include protease inhibitors (e.g., Thermo Scientific™ Halt™ Protease Inhibitor Single-Use Cocktail, EDTA-free, Product No. 78425) when preparing cell lysates.
- Binding capacity and elution recovery will vary depending on the c-Myc-fusion protein and the elution method.
- A low-pH elution may be used for single-use applications. Optimal incubation time for low-pH elution is 5-10 minutes; exceeding 10 minutes may result in nonspecific binding and yield reduction. The c-Myc antibody will not leach from the beads when eluting with the recommended acidic elution buffer (0.1M glycine, pH 2.0).
- Basic elution buffer (e.g., 50mM NaOH) may be used to elute c-Myc-tagged protein; however, the stringency of the buffer will cause the c-Myc antibody to leach from the beads.
- If a gentle elution of c-Myc-tagged protein is desired, a competitive elution can be performed using 0.5mg/mL of Thermo Scientific™ Pierce™ c-Myc Peptide (Product No. 20170).
- Pierce Anti-c-Myc Magnetic Beads are compatible with IP and Western blot analysis.
- Do not use cell lysate containing dithiothreitol (DTT). DTT may cause the c-Myc antibody to leach from the beads.
- If desired, a reference c-Myc-tagged positive control is available (Product No. 23633).

Additional Materials Required

- Lysis Buffer: Buffer used to prepare cell lysate (e.g., Thermo Scientific™ Pierce™ IP Lysis Buffer, Product No. 87788, for mammalian cells or Thermo Scientific™ B-PER™ Bacterial Protein Extraction Reagent, Product No. 78243, for bacterial cells)

  Note: Use 1X TBS-T buffer (25mM Tris, 0.15M NaCl, 0.05% Tween-20 Detergent) to adjust the IP reaction volume.
- Wash Buffer: 5X TBS-T buffer (125mM Tris, 0.75M NaCl, 0.25% Tween-20 Detergent) (e.g., dilute 1:4 Thermo Scientific™ Pierce™ 20X TBS Tween-20 Buffer, Product No. 28360, with ultrapure water)
- Elution Buffer options:
  - IgG Elution Buffer, pH 2.0 (Product No. 21028) or 0.1M glycine, pH 2.0
  - 50mM NaOH
  - Pierce c-Myc-Peptide (Product No. 20170), 0.5 mg/mL
  - SDS-PAGE Sample Buffer (e.g., Thermo Scientific™ Lane Marker Non-Reducing Sample Buffer (5X), Product No. 39001)

    Note: Reducing sample buffer will result in loss of some antibody heavy and light chains from the beads.
- Neutralization Buffer: 1M Tris, pH 8.5
- Sample containing c-Myc-tagged protein

For Automated IP:

- 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)
- KingFisher Flex 96 Tip Comb for Deep Well Magnets (Product No. 97002534)

For Manual IP:

- 1.5mL microcentrifuge tubes
- Magnetic stand (e.g., Thermo Scientific™ MagnaBind™ Magnet for 6 × 1.5mL microcentrifuge tubes, Product No. 21359)
- End-over-end rocker or rotator

Procedure for IP of c-Myc-Tagged Proteins

A. Manual Immunoprecipitation

Note: The amount of lysate needed and incubation times required are dependent on the expression level of the c-Myc-tagged protein and require optimization for each specific system. For co-immunoprecipitation (co-IP), buffers must be optimized to maintain the protein: protein interaction.

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

1. Place 25-100µL of Pierce Anti-c-Myc Magnetic Beads into a 1.5mL microcentrifuge tube.
2. Add 175µL of 1X TBS-T buffer (25mM Tris, 0.15M NaCl, 0.05% Tween-20 Detergent) 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 1X TBS-T buffer to the tube. Invert the tube several times or gently vortex to mix for 1 minute. Collect beads with a magnetic stand. Remove and discard the supernatant.
5. Add the sample containing c-Myc-tagged protein (~100µg of protein in 100µL) to the pre-washed magnetic beads, add 400µL of 1X TBS-T buffer and incubate at room temperature for 30 minutes with mixing.
6. Collect the beads with a magnetic stand, remove the unbound sample and save for analysis.
7. Add 300µL of 5X TBS-T buffer (125mM Tris, 0.75M NaCl, 0.25% Tween-20 Detergent) to the tube and gently mix. Collect the beads and discard the supernatant. Repeat this wash twice.
8. Add 300µL of ultrapure water to the tube and gently mix. Collect the beads on a magnetic stand and discard the supernatant.

B. Elution of c-Myc-Tagged Protein

Note: Select one of the elution protocols below. If the eluted c-Myc-tagged protein will be used for function applications or is sensitive to pH extremes, elute the protein with Pierce c-Myc Peptide.

Gentle Elution Protocol

1. Prepare Pierce c-Myc Peptide at 0.5mg/mL in TBS.
2. Add 100µL of 0.5mg/mL Pierce c-Myc Peptide to the beads, gently vortex to mix and incubate the sample at 37°C on a rotator for 5-10 minutes. Elution may be performed at reduced temperatures, but lower yields may result.
3. Separate the beads on a magnetic stand and save the supernatant containing the target antigen.
4. Repeat elution step once for higher recovery.

Chemical Elution Protocols

- **Elution Using 50mM NaOH (Basic Elution)**

1. Add 100µL of 50mM NaOH to the tube.
2. Gently vortex to mix and incubate the sample at room temperature on a rotator for 5 minutes.
3. Magnetically separate the beads and save the supernatant containing the target antigen.
4. To neutralize the sample, add 50µL of Neutralization Buffer for each 100µL of eluate.

- **Elution Using IgG Elution Buffer (Acidic Elution)**

1. Add 100µL of IgG Elution Buffer, pH 2.0 or 0.1M glycine, pH 2.0.
2. Gently vortex to mix and incubate the sample at room temperature on a rotator for 5-10 minutes.
3. Magnetically separate the beads and save the supernatant containing the target antigen.
4. To neutralize the low pH, add 15µL of Neutralization Buffer for each 100µL of eluate.

- **Elution Using Sample Buffer**

1. Add 100µL of SDS-PAGE Sample Buffer to the tube.
2. Gently vortex to mix and incubate the sample at 95-100°C for 5-10 minutes.
3. Magnetically separate the beads and save the supernatant containing the target antigen.

   Note: Using non-reducing sample buffer can minimize interference from co-eluting antibody fragments.

   Note: If elution under reducing conditions is desired, add 2.5µL of 2M DTT to the 100µL sample.

C. Automated Immunoprecipitation and Elution

Note: This 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 "c-Myc-Tag-Immunoprecipitation" protocol from the Thermo Scientific website (http://www.thermoscientific.com/bindit-protocols) into the BindIt Software on an external computer.
2. Transfer the protocol to the KingFisher Flex Instrument 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 c-Myc tag IP protocol using the Thermo Scientific Microtiter Deep Well 96 Plates.

Plate #	Plate Name	Content	Volume	Time/Speed
1	Beads	Anti-c-Myc magnetic beads	25µL	5 seconds
		1X TBS-T	175µL	
2	Bead Wash	1X TBS-T	1000µL	1 minute/slow
3	Bind	Sample containing c-Myc-tagged protein	300µL	30 minutes/slow
4	Wash 1	5X TBS-T	300µL	30 seconds/slow
5	Wash 2	5X TBS-T	300µL	30 seconds/slow
6	Wash 3	Ultrapure water	300µL	30 seconds/slow
7	Elution	Elution Buffer	100µL	10 minutes/slow
8	Tip Plate	KingFisher 96 Tip Comb for Deep Well Magnets	-	10 seconds/fast

Notes:

- If less 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.

- If low-pH Elution Buffer is selected for elution, neutralize the pH by adding 15µL of Neutralization Buffer for each 100µL of eluate upon run completion.
- If using SDS-PAGE Sample Buffer in a heated elution, install the KingFisher Flex Heating Block (see manual for proper installation) to heat samples at 95-100°C for 5-10 minutes.

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 of the plates are removed.

Troubleshooting

Problem	Possible Cause	Solution
Little or no c-Myc-tagged protein is detected	Tagged protein degraded	Include protease inhibitors (e.g., Product No. 78425 or 78430) in the lysis buffer
		Use new lysate or lysate stored at -80°C
	No or minimal tagged protein was expressed	Verify protein expression by SDS-PAGE or Western blot analysis of the lysate using an c-Myc-tagged positive control as a reference
		Increase the amount of lysate used for IP/Co-IP
		Use a more sensitive detection system such as Thermo Scientific™ SuperSignal™ West Femto Chemiluminescent Substrate (Product No. 34095)
Magnetic beads aggregated	Magnetic beads were frozen or centrifuged	Handle the beads as directed in the instructions
	Buffer was incompatible with magnetic beads	
	Detergent was not added to the wash and bind solutions	
Failure to co-IP interacting protein	Wash conditions were too stringent for the weak or transient interaction	Reduce the number of washes
		Lower the ionic strength of the wash buffer
	Interacting protein was expressed at a low level	Apply additional protein sample
		Use a more sensitive detection system
	Buffer system was not optimal for the protein: protein interaction	Optimize the co-IP buffer
	Insufficient sample was loaded on the gel for Western blot detection	Elute sample in 30% acetonitrile/0.5% formic acid, then dry down using a Thermo Scientific™ Speedvac™ Vacuum Concentrator. Bring the sample back up in SDS-PAGE Sample Buffer and load entire elution fraction on to gel

Additional Information Available on Our Website

- Frequently Asked Questions
- Tech Tip #43: Protein stability and storage
- Visit *www.thermoscientific.com/kingfisher* for information on the 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 the U.S.A.

Frequently Asked Questions for the KingFisher Instrument

Question	Answer
Which plates are compatible with KingFisher Flex Instruments?	The KingFisher Flex Instrument is compatible with the KingFisher 24 Deep Well Plates, Microtiter Deep Well 96 Plates and 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 may 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 and the eluted proteins stick to the wells?	Eluted proteins and proteins conjugated to beads can nonspecifically bind to plastics. Adding detergent (e.g., 0.05%-0.1% Tween-20 Detergent) to the Binding/Wash Buffer prevents the protein conjugated to the bead from sticking. Also, including a small amount of detergent in the elution buffer (e.g., 0.05% Tween-20 Detergent) or silanizing the elution plate can minimize sticking
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

88838	**Pierce c-Myc-Tag Magnetic IP/Co-IP Kit**
88831-2	**HisPur™ Ni-NTA Magnetic Beads**
88821-2	**Pierce Glutathione Magnetic Beads**
88836-7	**Pierce Anti-HA Magnetic Beads**
MA1980	**Anti-c-Myc Antibody**
20170	**Pierce c-Myc Peptide**
20290	**DTT (Dithiothreitol)**
78260	**B-PER II Bacterial Protein Extraction Reagent**
87788	**Pierce IP Lysis Buffer**
28360	**20X TBS Tween-20 Buffer**
39000	**Lane Marker Reducing Sample Buffer (5X)**
39001	**Lane Marker Non-Reducing Sample Buffer (5X)**
82033	**Pierce Agarose qIP Protein Interaction Kit, Tluc and Myc tags**
82036	**Pierce Magnetic qIP Protein Interaction Kit, Tluc and Myc tags**

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