INSTRUCTIONS

Pierce™ Anti-HA Magnetic Beads

88836 88837 2515.0

Number	Description
88836	**Pierce Anti-HA Magnetic Beads**, 1mL, supplied at 10mg/mL in PBS containing 0.05% Tween™-20 Detergent and 0.05% NaN_3
88837	**Pierce Anti-HA 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.

Table of Contents

Introduction

The Thermo Scientific Pierce Anti-HA Magnetic Beads are used for the immunoprecipitation (IP) of specific HA-tagged proteins expressed in human *in vitro* expression systems and bacterial and mammalian cell lysates. The anti-HA antibody coupled to the resin is a high-affinity mouse IgG_1 monoclonal antibody that recognizes the HA-epitope tag (YPYDVPDYA) derived from the human influenza hemagglutinin (HA) protein. For IP, the beads are added to a sample containing HA-tagged proteins. The bound, HA-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-HA 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-ERK-HA (70kDa fusion protein)/mg of beads

Important Product Information

- Do not centrifuge, dry or freeze the Pierce Anti-HA Magnetic Beads. Centrifuging, drying or freezing will 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 HA-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) in the preparation of cell lysates.
- Binding capacity and elution recovery will vary depending on the HA-fusion protein and the method of elution.
- 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 HA 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 HA-tagged protein; however, the stringency of the buffer will cause the HA antibody to leach from the beads.
- If a gentle elution of HA-tagged protein is desired, a competitive elution can be performed using 2mg/mL of Thermo Scientific Pierce HA Peptide (Product No. 26184).
- Pierce Anti-HA Magnetic Beads are compatible with immunoprecipitation and analyses by Western blot.
- Do not use cell lysate containing dithiothreitol (DTT). DTT may cause the HA antibody to leach from the beads.
- If desired, a reference HA-tagged positive control is available (Product No. 26180X).

Procedure for IP of HA-Tagged Proteins

A. Additional Materials Required

- 1.5mL microcentrifuge tubes
- Magnetic stand or KingFisher™ Instrument
- Binding Buffer: Buffer used to prepare cell lysate (e.g., Thermo Scientific 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 Binding/Lysis Buffer to adjust the IP reaction volume.
- Wash Buffer: Tris-buffered saline (TBS, Product No. 28360) containing 0.05% Tween-20 Detergent (TBS-T)
- Elution Buffer options:
  - IgG Elution Buffer, pH 2.0 (Product No. 21028) or 0.1M glycine, pH 2.0
  - 50mM NaOH
  - Thermo Scientific Pierce HA Peptide (Product No. 26184), 2mg/mL
  - SDS-PAGE Sample Buffer (e.g., Thermo Scientific Lane Marker Non-Reducing Sample Buffer (5X), Product No. 30001)

    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 HA-tagged protein
- End-over-end rocker or rotator

B. Manual Immunoprecipitation

Note: The amount of lysate needed and incubation times are dependent on the expression level of the HA-tagged protein and require optimization for each specific system. For a 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 25µL (0.25mg) of Pierce Anti-HA Magnetic Beads into a 1.5mL microcentrifuge tube.

2. Add 175µL of 0.05% TBS-T 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 TBS-T 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 sample containing HA-tagged protein to the pre-washed magnetic beads 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 TBS-T 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.

C. Elution of HA-Tagged Protein

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

Gentle Elution Protocol

1. Prepare Pierce HA Peptide at 2mg/mL in TBS.
2. Add 100µL of 2mg/mL Pierce HA 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

- **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. Neutralize the sample by adding 50µL of Neutralization Buffer for each 100µL of eluate.

- **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.

- **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.

D. Automated Immunoprecipitation and Elution

Note: This protocol is designed for use with the KingFisher Flex or KingFisher 96 Instrument. The protocol can be modified according to your needs using the Thermo Scientific BindIt Software provided with the instrument.

1. Download the "HA_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 or KingFisher 96 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 HA-tag IP protocol using the Thermo Scientific Microtiter Deep Well 96 Plates.

Plate #	Plate Name	Content	Volume	Time/Speed
1	Beads	Anti-HA magnetic beads	25µL	5 seconds
		TBST	175µL	
2	Bead Wash	TBST	1000µL	1 minute/slow
3	Bind	Sample containing HA-tagged protein	300µL	30 minutes/slow
4	Wash 1	TBST	300µL	30 seconds/slow
5	Wash 2	TBST	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 or 96 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 or KingFisher 96 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 HA-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 HA-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)
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
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	

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 VWR. 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 and KingFisher 96 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 or the eluted proteins stick to the wells?	Eluted proteins and proteins conjugated to beads can nonspecifically bind to plastics. Adding detergent to the Binding/Wash Buffer prevents the protein conjugated to the bead from sticking (e.g., 0.05%-0.1% Tween-20 Detergent). Also include a small amount of detergent in the elution buffer (e.g., 0.05% Tween-20 Detergent) or silanize the elution plate
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 HA-Tag Magnetic IP/Co-IP Kit**
88832-3	**HisPur™ Ni-NTA Magnetic Beads**
88821-2	**Pierce Glutathione Magnetic Beads**
26183	**Anti-HA Antibody**
26184	**Pierce HA Peptide**
20290	**DTT (Dithiothreitol)**
78260	**B-PER™ II Bacterial Protein Extraction Reagent**
87788	**Pierce IP Lysis Buffer**
39000	**Lane Marker Reducing Sample Buffer (5X)**
39001	**Lane Marker Non-Reducing Sample Buffer (5X)**

Tween is a trademark of Croda International Plc.

Products are warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation"). No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than Buyer. Any model or sample furnished to Buyer is merely illustrative of the general type and quality of goods and does not represent that any Product will conform to such model or sample.
NO OTHER WARRANTIES, EXPRESS OR IMPLIED, ARE GRANTED, INCLUDING WITHOUT LIMITATION, IMPLIED WARRANTIES OF MERCHANTABILITY, FITNESS FOR ANY PARTICULAR PURPOSE, OR NON INFRINGEMENT. BUYER'S EXCLUSIVE REMEDY FOR NON-CONFORMING PRODUCTS DURING THE WARRANTY PERIOD IS LIMITED TO REPAIR, REPLACEMENT OF OR REFUND FOR THE NON-CONFORMING PRODUCT(S) AT SELLER'S SOLE OPTION. THERE IS NO OBLIGATION TO REPAIR, REPLACE OR REFUND FOR PRODUCTS AS THE RESULT OF (I) ACCIDENT, DISASTER OR EVENT OF FORCE MAJEURE, (II) MISUSE, FAULT OR NEGLIGENCE OF OR BY BUYER, (III) USE OF THE PRODUCTS IN A MANNER FOR WHICH THEY WERE NOT DESIGNED, OR (IV) IMPROPER STORAGE AND HANDLING OF THE PRODUCTS.

Unless otherwise expressly stated on the Product or in the documentation accompanying the Product, the Product is intended for research only and is not to be used for any other purpose, including without limitation, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses, or any type of consumption by or application to humans or animals.

Current product instructions are available at *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.

© 2012 Thermo Fisher Scientific Inc. All rights reserved. Unless otherwise indicated, all trademarks are property of Thermo Fisher Scientific Inc. and its subsidiaries. Printed in the USA.