INSTRUCTIONS

Pierce™ NHS-Activated Magnetic Beads

88826 88827 2320.2

Number	Description
88826	**Pierce NHS-Activated Magnetic Beads,** 1mL, supplied at 10mg/mL in *N*,*N*-dimethylacetamide (DMAC)
88827	**Pierce NHS-Activated Magnetic Beads,** 5mL, supplied at 10mg/mL in *N*,*N*-dimethylacetamide (DMAC)

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

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Introduction

The Thermo Scientific™ Pierce™ NHS-Activated Magnetic Beads covalently immobilize proteins for the affinity purification of antibodies, antigens and other biomolecules. The activated magnetic beads contain *N*-hydroxysuccinimide (NHS) functional groups, which react with primary amines on proteins or other molecules to form stable amide linkages. The coupling reaction is performed in an amine-free buffer at pH 7-9. The beads are manually removed from the solution using a magnetic stand or by automation using an instrument such as the Thermo Scientific™ KingFisher™ Flex System. Automated instruments are especially useful for large screening of multiple samples.

Table 1. Characteristics of Thermo Scientific Pierce NHS-Activated Magnetic Beads.

Composition:	*N*-hydroxysuccinimide (NHS) functional groups on a blocked magnetic bead surface
Magnetization:	Superparamagnetic (no magnetic memory)
Mean Diameter:	1µm (nominal)
Density:	2.0g/cm^3
Bead Concentration:	10mg/mL in DMAC
Binding Capacity:	≥ 26µg of rabbit IgG/mg of beads

Important Product Information

- Magnetic beads are moisture-sensitive. To protect the beads, cap the bottle immediately after removing the slurry and wrap lab film around the cap before storing at 4°C.
- Do not centrifuge, dry or freeze the magnetic beads. Bead aggregation and loss of binding activity can result from using these methods.

- Estimate the amount of protein coupled to the magnetic beads with a protein assay (e.g., Thermo Scientific™ Pierce™ 660nm Protein Assay, Product No. 22660 and 22662) and subtract the amount of flow-through protein from the loaded protein. To measure the amount of protein on the bead directly, use the Thermo Scientific™ Pierce™ Micro BCA Protein Assay (Product No. 23235, see Tech Tip #75 from our website).
- For coupling antibodies to magnetic beads, ensure the antibody storage solution does not contain a protein stabilizer (e.g., BSA, gelatin), which inhibits coupling of the antibody to the beads. Protein stabilizers can be removed using the Thermo Scientific Pierce Antibody Clean-up Kit (Product No. 44600, see Tech Tip #55 from our website). For best results, buffer exchange the antibody into 50mM borate, pH 8.5 (e.g., Thermo Scientific™ BupH™ Borate Buffer Packs, Product No. 28384).
- Primary amine-containing buffers (e.g., Tris and glycine) inhibit coupling of protein to the magnetic beads. Remove primary amine-containing buffer using dialysis (e.g., Thermo Scientific™ Slide-A-Lyzer™ G2 Dialysis Cassettes, 10K MWCO, 3mL, Product No. 87730) or desalting (e.g., Thermo Scientific™ Zeba™ Spin Desalting Columns, 7K MWCO, 0.5mL, Product No. 89882).
- The coupling efficiency of protein to the magnetic beads varies depending on the specific protein. Typically, 0.1-2.0mg/mL of protein produces optimal protein coupling; however, optimize the concentration for each specific protein. As a reference, binding capacities of different proteins are listed in Table 2.

Table 2. Binding capacity of proteins with different molecular weights on the Thermo Scientific Pierce NHS-Activated Magnetic Beads.

Protein	Molecular weight (kDa)	Binding capacity of NHS beads (µg/mg of bead)
IgG	150	50
Streptavidin	53	24
Protein A/G	50	21
Protein L	36	39

Note: Results will vary depending on the number of accessible primary and secondary amines.

Procedure for Protein Immobilization

Note: The following coupling procedure is for 300µL of magnetic beads in a 1.5mL microcentrifuge tube; scale the procedure as needed.

A. Additional Materials Required

- Wash Buffer A: ice-cold 1mM hydrochloric acid
- Coupling Buffer: 50mM borate, pH 8.5 (Product No. 28384) or other amine-free buffer, pH 7-9
- Protein Solution: 0.1-2.0mg/mL in Coupling Buffer. For proteins already in solution, completely remove primary amine-containing buffer (e.g., Tris or glycine) using desalting or dialysis.
- Quenching Buffer: 3M ethanolamine, pH 9.0
- Storage Buffer: Coupling Buffer with 0.05% sodium azide
- 1.5mL microcentrifuge tubes
- Ultrapure water
- Wash Buffer B: 0.1M glycine, pH 2.0

For Manual Coupling:

- Magnetic stand (e.g., Thermo Scientific Magnabind Magnet for 6 × 1.5mL microcentrifuge tubes, Product No. 21359)

For Automated Coupling:

- 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

B. Manual Procedure for Coupling and Blocking

1. Equilibrate protein solution and magnetic beads to room temperature.

   Note: To ensure homogeneity, mix the magnetic beads thoroughly before use by repeated inversion, gentle vortexing or using a rotating platform.
2. Place 300µL of magnetic beads into a 1.5mL microcentrifuge tube.
3. Place the tube into a magnetic stand, collect the beads and discard the supernatant.
4. Add 1mL of ice-cold Wash Buffer A into the tube and gently vortex for 15 seconds to mix.
5. Place the tube into a magnetic stand, collect the beads and discard the supernatant.

   Note: Immediately proceed with adding the protein solution.
6. Add 300µL of protein solution into the tube and vortex for 30 seconds.
7. Incubate the tube for 1-2 hours at room temperature on a rotator. During the first 30 minutes of the incubation, vortex the tube for 15 seconds every 5 minutes. For the remaining time, vortex the tube for 15 seconds every 15 minutes until incubation is complete.

   Note: If required, incubate overnight at 4°C.
8. Collect the beads with a magnetic stand and save the flow-through.
9. Add 1mL of Wash Buffer B to the beads and vortex the tube for 15 seconds.
10. Place the tube into a magnetic stand, collect the beads and discard the supernatant.
11. Repeat Steps 9 and 10 one time.
12. Add 1mL of ultrapure water to the beads and vortex for 15 seconds.
13. Place the tube into a magnetic stand, collect the beads and discard the supernatant.
14. Add 1mL of Quenching Buffer to the beads and vortex the tube for 30 seconds.
15. Incubate the tube for 2 hours at room temperature on a rotator.
16. Place the tube into a magnetic stand, collect the beads and discard the supernatant.
17. Add 1mL of purified water to the tube, mix well, collect the beads with a magnetic stand and discard the supernatant.
18. Add 1mL of Storage Buffer to the tube, mix well, collect the beads with a magnetic stand and discard the supernatant. Repeat this wash two additional times.
19. Add 300µL of Storage Buffer to the beads, mix well and store at 4°C until ready for use.

    Note: The final concentration of the protein-coupled magnetic beads is 10mg/mL.

C. Automated Procedure for Coupling and Blocking

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 “Protein Coupling” protocol from the Thermo Fisher 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 3.

Table 3. Pipetting instructions for the Coupling and Blocking protocol using the Microtiter Deep Well 96 Plates.

Plate #	Plate Name	Content	Volume	Time/Speed
1	Beads	NHS-Activated Beads	300µL	Collect Beads
2	Wash 1	Wash Buffer A	1mL	10 seconds/Slow
3	Coupling	Protein Sample in Coupling Buffer	300µL	1-2 hours/Slow
4	Wash 2	Wash Buffer B	1mL	10 seconds/Slow
5	Wash 3	Wash Buffer B	1mL	10 seconds/Slow
6	Wash 4	Purified Water	1mL	10 seconds/Slow
7	Quench	Quenching Buffer	1mL	2 hours/Slow
8	Wash 5	Purified Water	1mL	30 seconds/Slow
9	Wash 6	Storage Buffer	1mL	30 seconds/Slow
10	Storage	Storage Buffer	300µL	Release Beads
11	Tip Plate	KingFisher Flex 96 Tip Comb for Deep Well Magnets	-	-

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 the Tip Plate (Plate #11) and press Start. The instrument places the Tip Comb onto the magnet head.
7. Remove the Tip Plate.
8. Load Plates #1-8 into the instrument according to the protocol requests; place each plate in the same orientation. Confirm each action by pressing Start.
9. After sample processing through Plate #8, the instrument will pause and instruct to remove each individual processed plate while simultaneously loading the remaining three plates, Plates #9-11. For example, remove plate #1 and load plate #9 into that position. Confirm each action by pressing Start.
10. After sample processing, remove the plates as instructed by the instrument display. Press Start after each plate. Press Stop after removing all of the plates.

Notes:

- Load ice-cold 1mM Wash Buffer A in Plate #2 immediately before instrument loading to ensure the buffer remains cold.
- If using fewer than 96 wells, fill the same wells in each plate. For example, if using wells A1 through A12, use these same wells in all plates.
- Combine the Tip Comb with a Deep Well 96 Plate. See the instrument user manual for detailed instructions.

Procedure for Immunoprecipitation

A. Additional Materials Required

- 1.5mL microcentrifuge tubes
- Binding/Wash Buffer: Tris-buffered saline (TBS, Product No. 28360) containing 0.05% Tween 20 Detergent. (Optional: To reduce nonspecific binding and background, increase the concentration of NaCl from 0.15M to 0.5M.)
- Low-pH Elution Buffer: 0.1M glycine, pH 2.0
- Antibody for immunoprecipitation
- Antigen sample for immunoprecipitation (e.g., cell lysate)
- Neutralization Buffer: High-ionic strength alkaline buffer such as a 1M phosphate or 1M Tris; pH 7.5-9
- Optional: Protease inhibitor cocktail (e.g., Thermo Scientific Halt Protease Inhibitor Single-Use Cocktail EDTA-free, Product No. 78425)

For Manual IP:

- Magnetic stand (e.g., MagnaBind Magnet for 6 × 1.5mL Microcentrifuge Tubes, Product No. 21359)

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)

B. Manual Antigen Immunoprecipitation

1. Add 25µL (0.25mg) of antibody-coupled magnetic beads to a 1.5mL centrifuge tube.
2. Place tube in a magnetic stand, collect the beads and discard the supernatant.
3. Dilute antigen sample for immunoprecipitation to 1-2mg/mL using Binding/Wash Buffer.
4. Add 500µL of diluted antigen sample to the tube containing antibody-coupled magnetic beads and incubate for 1-2 hours at room temperature on a rotator or mixer. Gently vortex the beads every 10-15 minutes during incubation to ensure the beads remain in suspension.
5. Collect the beads with a magnetic stand, remove the unbound sample and save for analysis.
6. Add 500µL of Binding/Wash Buffer to the tube and gently mix. Collect the beads on a magnetic stand and discard the supernatant. Repeat this step one time.
7. Add 500µL of ultrapure water to the tube and gently mix. Collect the beads on a magnetic stand and discard the supernatant.
8. Add 100µL of Elution Buffer to the tube. Incubate for 5 minutes at room temperature on a rotator or mixer. Magnetically separate the beads and save the supernatant containing the target antigen.

   Note: One elution may be sufficient; however, optimization is required for each system.
9. Repeat Step 8 and combine the two eluates. To neutralize the low pH of the solution, add 10µL of Neutralization Buffer for each 100µL of eluate.

C. Automated Antigen Immunoprecipitation

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

1. Download the "NHS_IP" protocol from the Thermo Fisher 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 4.

Table 4. Pipetting instructions for the IP protocol using Microtiter Deep Well 96 Plates.

Plate #	Plate Name	Content	Volume	Time/Speed
1	Bind	Beads	25µL	2 hour/Slow
		Antigen Sample for IP	500µL	
2	Wash 1	Binding/Wash Buffer	500µL	30 seconds/Slow
3	Wash 2	Binding/Wash Buffer	500µL	30 seconds/Slow
4	Wash 3	Ultrapure Water	500µL	30 seconds/Slow
5	Elution 1	Elution Buffer	100µL	5 minutes/Slow
6	Elution 2	Elution Buffer	100µL	5 minutes/Slow
7	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 requests, placing each plate in the same orientation. Confirm each action by pressing Start.
7. After sample processing, remove the plates as instructed by the instrument's display. Press Start after each plate. Press Stop after removing all of the plates.

Notes:

- The low-pH elutions must be neutralized by adding 10µL of Neutralization Buffer for each 100µL of eluate directly to each well immediately after incubation.
- 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.
- Combine the Tip Comb with a Deep Well 96 Plate. See the instrument user manual for detailed instructions.

Troubleshooting

Problem	Possible Cause	Solution
Low coupling efficiency	Primary amine-containing buffer was not completely removed before coupling	Dialyze or desalt sample to completely remove Tris and glycine
	Protein addition was delayed	Immediately mix protein with beads after washing
Protein is not soluble in coupling buffer	Molecule was hydrophobic	Dissolve protein in coupling buffer containing up to 20% DMSO
Beads aggregate during the coupling process	Proteins on bead surface adhered to tube plastic	After blocking, add 0.05% detergent (e.g., Tween-20 Detergent) to the water wash and the Storage Buffer (Part C) **Note:** Do not use detergent in the coupling step

Additional Information Available on Our Website

- Frequently Asked Questions
- Tech Tip #43: Protein stability and storage
- Tech Tip #55: Remove BSA and gelatin from antibody solutions using Melon Gel
- Tech Tip #75: Measure protein bound to Pierce NHS-Activated Magnetic Beads
- Visit *www.thermoscientific.com/kingfisher* for information on KingFisher Products.
- In the U.S.A., purchase KingFisher Supplies from Fisher Scientific. Outside the U.S.A., contact your local Thermo Fisher Scientific office to purchase KingFisher Supplies.

Related Thermo Scientific Products

88828	**Pierce Direct Magnetic IP/Co-IP Kit**
88802-3	**Pierce Protein A/G Magnetic Beads**
88845-6	**Pierce Protein A Magnetic Beads**
88847-8	**Pierce Protein G Magnetic Beads**
88804	**Pierce Classic Magnetic IP/Co-IP Kit**
88805	**Pierce Crosslink Magnetic IP/Co-IP Kit**
88816-7	**Pierce Streptavidin Magnetic Beads**
88821-2	**Pierce Glutathione Magnetic Beads**
23235	**Micro BCA Protein Assay Kit**
21030	**Gentle Ag/Ab Binding and Elution Buffer Kit**
44600	**Pierce Antibody Clean-up Kit**

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