

# Pierce<sup>®</sup> Protein A and Protein G Magnetic Beads

88800 88801 88806 88807

2075.0

Number	Description
88800	<b>Pierce Protein A Magnetic Beads</b> , 1 ml, supplied in PBS with 1% BSA and 0.1% NaN <sub>3</sub>
88801	<b>Pierce Protein A Magnetic Beads</b> , 5 ml, supplied in PBS with 1% BSA and 0.1% NaN <sub>3</sub>
88806	<b>Pierce Protein G Magnetic Beads</b> , 1 ml, supplied in PBS with 1% BSA and 0.1% NaN <sub>3</sub>
88807	<b>Pierce Protein G Magnetic Beads</b> , 5 ml, supplied in PBS with 1% BSA and 0.1% NaN <sub>3</sub>

**Storage:** Upon receipt store at 4°C. Products are shipped on an ice pack.

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## Introduction

The Thermo Scientific Pierce Magnetic Beads provide a fast and convenient method for manual and automated magnetic isolation of antibodies and antigens using affinity binding. Protein A and Protein G Magnetic Beads are typically used for isolating antibodies from serum, cell culture supernatants or ascites and for immunoprecipitating antigens from cell or tissue extracts. For antibody purification, the beads are incubated with the antibody solution and then magnetically separated from the supernatant. For immunoprecipitation, the beads are added to an antigen sample that was pre-incubated with antibody. The antibody-antigen complex is magnetically removed from the sample. The bound antibodies and/or antigens are dissociated from the beads using an elution buffer. For manual processing a magnetic stand is used (see Related Products), or an automated magnetic platform is used, such as the KingFisher 96 and KingFisher Flex Instruments. These instruments are especially useful for large-scale screening of multiple samples.

**Table 1. Characteristics of Thermo Scientific Pierce Protein A and Protein G Magnetic Beads.**

Composition:	Iron oxide
Magnetization:	Superparamagnetic (no magnetic memory)
Mean Diameter:	1.5 μm
Density:	2.5 g/cm <sup>3</sup>
Surface Area:	> 100 m <sup>2</sup> /g
Bead Concentration:	10 mg/ml
Binding Capacity (Protein A):	≥ 0.4 mg rabbit IgG/ml
Binding Capacity (Protein G):	≥ 0.4 mg rabbit IgG/ml

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## Important Product Information

- The Pierce Magnetic Beads will aggregate and lose binding activity if frozen, dried or centrifuged.
- To minimize protein degradation, include protease inhibitors (e.g., Halt Protease Inhibitor Single-Use Cocktail EDTA-free, Product No. 78425) in cell lysate preparations.
- A low pH elution may be used for single-use applications. To limit leaching of Protein A or Protein G, do not exceed 10 minutes for the elution step in either the manual or automated protocol.
- Boiling the beads in SDS-PAGE sample buffer is acceptable for single-use applications. Boiling will cause bead aggregation and loss of binding activity.
- Pierce Protein A and Protein G Magnetic Beads are effective for antibody purification and immunoprecipitation with Western blot detection. Pierce Protein G Magnetic Beads are also effective for immunoprecipitation with detection by mass spectrometry.

## Procedure for Manually Pre-Washing Magnetic Beads

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

### Manual applications only:

1. Place 50  $\mu$ l (0.5 mg) of the magnetic beads into a 1.5 ml microcentrifuge tube.
2. Place the tube into a magnetic stand to collect the beads against the side of the tube. Remove and discard the supernatant.
3. Add a minimum of 300  $\mu$ l of Binding/Wash Buffer to the tube (for 0.5 to 3.0 mg, use 300-500  $\mu$ l; for > 3.0 mg, use 1 ml). Invert the tube several times or vortex gently to mix. Collect the beads with a magnetic stand and remove and discard the supernatant. Repeat this step twice.
4. Proceed to Procedure for Manual Antibody Purification or Procedure for Manual Immunoprecipitation below.

## Procedure for Manual Antibody Purification

### A. Additional Materials Required

- 1.5 ml microcentrifuge tubes
- Serum, concentrated cell culture supernatant or concentrated ascites (Thermo Scientific iCON™ Concentrator 20 ml/20K, Product No. 89887)
- Binding/Wash Buffer: Tris-buffered saline (TBS, Product No. 28379) containing 0.1% Tween®-20 Detergent
- Elution Buffer: IgG Elution Buffer (Product No. 21004 or 21009) or 0.1 M glycine, pH 2-3
- Neutralization Buffer: High-ionic strength alkaline buffer such as 1 M phosphate or 1 M Tris; pH 7.5-9
- Magnetic stand (e.g., Thermo Scientific MagnaBind Magnet for 6  $\times$  1.5 ml Microcentrifuge Tubes, Product No. 21359)

### B. Antibody purification from serum, cell culture supernatant or ascites

1. Dilute 10  $\mu$ l of serum, concentrated cell culture supernatant or ascites in 490  $\mu$ l of Binding/Wash Buffer.  
**Note:** Modify the sample volume as needed. If the sample is less than 500  $\mu$ l, dilute it to 500  $\mu$ l with Binding/Wash Buffer.
2. Add diluted serum, cell culture supernatant or ascites to the tube containing the pre-washed magnetic beads from above and gently invert or vortex to mix.
3. Incubate the samples at room temperature with mixing for 1 hour.
4. Collect the beads with a magnetic stand and remove and discard the supernatant.
5. Add 500  $\mu$ l of Binding/Wash Buffer to the tube, mix well, collect the beads with a magnetic stand and discard the supernatant. Repeat this wash twice.

- Add 100 µl of Elution Buffer to the tube, mix well and incubate 5 minutes at room temperature with occasional mixing.  
**Note:** If the elution is not complete, perform additional 5-minute elutions. Combine the eluates from multiple elutions.
- Collect the beads with a magnetic stand. Remove and save the supernatant that contains the eluted antibody. To neutralize the low pH, add 5 µl of Neutralization Buffer per 100 µl of eluate.

## Procedure for Automated Antibody Purification

### A. Additional Materials Required

- KingFisher Flex with 96 Deep Well Head (Product No. 5400630) or KingFisher 96 (Product No. 5400500) Instrument
- Microtiter Deep Well 96 Plate, V-bottom, Polypropylene (100-1,000 µl; Product No. 95040450)
- KingFisher Flex 96 Tip Comb for Deep Well Magnets (Product No. 97002534)
- Serum, concentrated cell culture supernatant or concentrated ascites (iCON Concentrator 20 ml/20K, Product No. 89887)
- Binding/Wash Buffer: Tris-buffered saline (TBS, Product No. 28379) containing 0.1% Tween-20 Detergent
- Elution Buffer: IgG Elution Buffer (Product No. 21004 or 21009) or 0.1 M glycine, pH 2-3
- Neutralization Buffer: High-ionic strength alkaline buffer such as 1 M phosphate or 1 M Tris; pH 7.5-9

### B. Preparation of the KingFisher Instrument and Plate Set-up

**Note:** The following protocol is for general use with the KingFisher Flex or KingFisher 96 Instrument. Modify the protocol as needed using the BindIt™ Software provided with the instrument.

- Download the Antibody Purification protocol from the Thermo Scientific web site ([www.thermo.com/kingfisher](http://www.thermo.com/kingfisher)) into the Thermo Scientific BindIt Software on an external computer.
- 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.
- Set up the plates according to Table 2.

**Table 2. Pipetting instructions for the antibody purification protocol.**

Plate #	Plate Name	Plate Type	Content	Volume
1	Beads	Microtiter Deep Well 96 Plate	Protein A/G beads	50 µl
			Binding/Wash Buffer	150 µl
2	Bead Wash	Microtiter Deep Well 96 Plate	Binding/Wash Buffer	1,000 µl
3	Bind	Microtiter Deep Well 96 Plate	Serum, concentrated ascites or concentrated cell culture supernatant	10 µl
			Binding/Wash Buffer	490 µl
4	Wash 1	Microtiter Deep Well 96 Plate	Binding/Wash Buffer	500 µl
5	Wash 2	Microtiter Deep Well 96 Plate	Binding/Wash Buffer	500 µl
6	Wash 3	Microtiter Deep Well 96 Plate	Binding/Wash Buffer	500 µl
7	Elution	Microtiter Deep Well 96 Plate	Elution Buffer	100 µl
8	Tip Plate	Microtiter Deep Well 96 Plate	KingFisher Flex 96 Tip Comb for Deep Well Magnets	–

### Notes:

- If using less 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.
- To ensure bead homogeneity, mix the vial thoroughly by repeated inversion, gentle vortexing or rotating platform before adding the beads to Plate 1.

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- Combine the Tip Comb with a Deep Well 96 plate. See the instrument user manual for detailed instructions.
  - Modify the sample volume as needed. If the sample is less than 500  $\mu$ l, dilute it to 500  $\mu$ l with Binding/Wash Buffer.

### C. Executing the Antibody Purification Protocol on the KingFisher Instrument

1. Select the protocol using the arrow keys in the instrument keypad and press *Start*. See the KingFisher Flex or KingFisher 96 User Manual for detailed information.
2. Slide open the door of the instrument's protective cover.
3. Load the plates into the instrument according to the protocol request, placing each plate in the same orientation. Confirm each action by pressing *Start*.
4. After the samples are processed, remove the plates as instructed by the instrument's display. Press *Start* after removing each plate.
5. Press *Stop* after all plates are removed.
6. Upon completion, if desired, neutralize the low pH by adding 5  $\mu$ l of Neutralization Buffer for each 100  $\mu$ l of eluate.

## Procedure for Manual Immunoprecipitation

### A. Additional Materials Required

- 1.5 ml microcentrifuge tubes
- Binding/Wash Buffer: Tris-buffered saline (TBS, Product No. 28379) containing 0.1% Tween-20 Detergent
- Elution Buffer: IgG Elution Buffer (Product No. 21004 or 21009) or 0.1 M glycine, pH 2-3
- Alternate Elution Buffer: SDS-PAGE reducing sample buffer
- Antibody for immunoprecipitation
- Antigen sample
- Cell Lysis Buffer (used to prepared antigen sample)
- Magnetic stand (e.g., Thermo Scientific MagnaBind Magnet for 6  $\times$  1.5 ml Microcentrifuge Tubes, Product No. 21359)

### B. Immunoprecipitation

**Note:** This protocol is a general guideline for immunoprecipitation and will require optimization for each experiment.

1. Combine antigen sample with 10  $\mu$ g of immunoprecipitation antibody. Incubate 1-2 hours at room temperature or overnight at 4°C with mixing.

**Note:** Adjust sample volume to at least 300  $\mu$ l with Cell Lysis Buffer or Binding/Wash Buffer.

2. Add the antigen sample/antibody mixture to a 1.5 ml microcentrifuge tube containing pre-washed magnetic beads (see above) and incubate at room temperature for 1 hour with mixing.
3. Collect the beads, remove the flow-through and save for analysis.
4. Add 300  $\mu$ l of Binding/Wash Buffer to the tube and gently mix. Collect the beads and then discard the supernatant. Repeat this wash twice.
5. Recover the antigen using one of the following elution methods:

**IgG Elution Buffer:** Add 100  $\mu$ l of Elution Buffer to the tube. Incubate the tube at room temperature with mixing for 5 minutes. Magnetically separate the beads and save the supernatant containing target antigen.

**SDS-PAGE Reducing Sample Buffer:** Add 100  $\mu$ l of SDS-PAGE reducing sample buffer to the tube and heat the samples at 96-100°C in a heating block for 5 minutes. Magnetically separate the beads and save the supernatant containing target antigen.

## Procedure for Automated Immunoprecipitation

### A. Additional Materials Required

- KingFisher Flex with 96 Deep Well Head (Product No. 5400630) or KingFisher 96 (Product No. 5400500) Instrument
- Thermo Scientific Microtiter Deep Well 96 Plate, V-bottom, polypropylene (Product No. 95040450)
- KingFisher Flex 96 Tip Comb for Deep Well Magnets (Product No. 97002534)
- 1.5 ml microcentrifuge tubes
- Binding/Wash Buffer: Tris-buffered saline (TBS, Product No. 28379) containing 0.1% Tween-20 Detergent
- Elution Buffer: IgG Elution Buffer (Product No. 21004 or 21009) or 0.1 M glycine, pH 2-3
- Alternative Elution Buffer: SDS-PAGE reducing sample buffer
- Antigen sample
- Antibody for immunoprecipitation

### B. Preparation of KingFisher Instrument and Plate Set-up

**Note:** The following protocol is for general use with the KingFisher Flex or KingFisher 96 Instrument. Modify the protocol as needed using the BindIt Software provided with the instrument.

1. Combine antigen sample with 10 µg of immunoprecipitation antibody per sample. Incubate for 1-2 hours at room temperature or overnight at 4°C with mixing.

**Note:** Step 1 is set up manually. After incubation the antigen/antibody mixture is transferred to the instrument.

**Note:** The automated protocol (Table 3) is for 300 µl of antigen sample/antibody mixture; however, 0.1-1 ml total sample volume per well may be used.

2. Download the “Immunoprecipitation low pH elution” or “Immunoprecipitation heated elution” protocol from our web site ([www.thermo.com/kingfisher](http://www.thermo.com/kingfisher)) into the BindIt Software on an external computer.
3. Transfer the protocol to the KingFisher Flex or KingFisher 96 Instrument from an external computer. See BindIt Software User Manual for detailed instructions on importing protocols.
4. Set up the plates according to Table 3.

**Table 3. Pipetting instructions for the immunoprecipitation protocol.**

Plate #	Plate Name	Plate Type	Content	Volume
1	Beads	Microtiter Deep Well 96 Plate	Protein A/G beads Binding/Wash Buffer	50 µl 150 µl
2	Bead Wash	Microtiter Deep Well 96 Plate	Binding/Wash Buffer	1,000 µl
3	Antigen Sample	Microtiter Deep Well 96 Plate	Antibody/Antigen sample	300 µl
4	Wash 1	Microtiter Deep Well 96 Plate	Binding/Wash Buffer	300 µl
5	Wash 2	Microtiter Deep Well 96 Plate	Binding/Wash Buffer	300 µl
6	Wash 3	Microtiter Deep Well 96 Plate	Binding/Wash Buffer	300 µl
7	Low pH elution	Microtiter Deep Well 96 Plate	Elution Buffer	100 µl
7	Heated Elution	Microtiter Deep Well 96 Plate	SDS-PAGE reducing sample buffer	100 µl
8	Tip Plate	Microtiter Deep Well 96 Plate	KingFisher Flex 96 Tip Comb for Deep Well Magnets	-

### Notes:

- If using less 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.

- 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 100 µl of 0.1 M glycine, pH 2-3 or 100 µl SDS-PAGE reducing sample buffer. If using the SDS-PAGE reducing sample buffer, install the heating block (see manual for proper installation).

### C. Executing the Immunoprecipitation Protocol on the KingFisher Flex/KingFisher 96 Instrument

1. Select the protocol using the arrow keys in the instrument keypad and press *Start*. See the instrument user manual for detailed information.
2. Slide open the door of the instrument's protective cover. Load the plates into the instrument according to the protocol request, placing each plate in the same orientation. Confirm each action by pressing *Start*.
3. After sample processing, remove plates as instructed by the instrument's display. Press *Start* after removing each plate.
4. Press *Stop* after all plates are removed.

## General Troubleshooting

Problem	Possible Cause	Solution
Low protein recovery	Proteolysis of sample	Add protease inhibitors
	Not enough magnetic beads used for capture	Increase the amount of magnetic beads used for capture
	Insufficient target protein present in sample	Increase amount of antigen sample
Protein does not elute	Elution conditions are too mild	Increase incubation time with elution buffer or use more stringent elution buffer
Multiple, nonspecific bands appear in eluted sample	Nonspecific protein binding to the magnetic beads	Add 50-200 mM NaCl to the Binding, Wash and/or Elution Buffers
Recovered protein is inactive	Elution conditions too stringent	Use a milder elution buffer (e.g. Gentle Elution Buffer, Product No. 21034)
Magnetic beads aggregate	Magnetic beads were frozen or centrifuged	Handle the beads as directed in the instructions
	Buffer is incompatible with magnetic beads	

## Frequently Asked Questions for the KingFisher Instruments

Question	Answer
Which plates are compatible with KingFisher Flex and KingFisher 96?	The KingFisher Flex Instrument is compatible with the KingFisher 24 Deep Well plates, Microtiter 96 Deep Well Plates, KingFisher 96 plates and 96 PCR plates. The KingFisher 96 Instrument is compatible with the Microtiter 96 Deep Well Plates, KingFisher 96 Plates 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 by 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.
Why do the beads stick to the plastic tips and wells or the eluted proteins sticks to the wells?	Proteins conjugated to beads and eluted proteins can nonspecifically bind to plastics. Adding detergent in the Binding/Wash Buffer prevents the protein conjugated to the beads from sticking (0.05%-0.1% Tween-20). Also include a small amount of detergent in the Elution Buffer (e.g., 0.05% Tween-20) 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.

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## Additional Information

- Visit [www.thermo.com/pierce](http://www.thermo.com/pierce) for additional information including the following:
  - Tech Tip protocol: Binding Characteristics for Immunoglobulins and Protein L, A, G and A/G
  - Tech Tip protocol: Protein Stability and Storage
- Visit [www.thermo.com/kingfisher](http://www.thermo.com/kingfisher) for information on 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.

## Related Products

24615	Imperial™ Protein Stain, 1 L, sufficient for up to 50 mini gels
34075	SuperSignal® West Dura Extended Duration Substrate, sufficient for 1,000 cm <sup>2</sup> of membrane
25200-25244	Precise™ Protein Gels, see catalog or web site for a complete listing
88821, 88822	Pierce Glutathione Magnetic Beads, 4 ml, 20 ml
88811, 88812	Pierce Magnetic TiO <sub>2</sub> Phosphopeptide Enrichment Kit
78428	Halt™ Phosphatase Inhibitor Single-Use Cocktail

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Current versions of product instructions are available at [www.thermo.com/pierce](http://www.thermo.com/pierce). For a faxed copy, call 800-874-3723 or contact your local distributor.

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