Thermo SCIENTIFIC

PRODUCT INFORMATION SHEET

Pierce™ Protein A/G Magnetic Agarose Beads

Catalog Numbers 78609 and 78610

Doc. Part No. 2162620 **Pub. No.** MAN0015959 **Rev.** A.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

The Thermo Scientific™ Pierce™ Protein A/G Magnetic Agarose Beads (Table 1) provide a fast, convenient method for purification of immunoglobulins from serum, cell culture supernatant, or ascites. For antibody purification, the beads are incubated with the antibody sample and then magnetically separated from the supernatant. Nonspecifically bound serum or host cell protein can be washed away before dissociating bound antibody with 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 Magnetic Particle Processor. Automated instruments are especially useful for higher throughput purification and screening of purification conditions.

Pierce™ Protein A/G Magnetic Agarose Beads contain a recombinant 50.5 kDa Protein A/G covalently attached to a magnetite-embedded agarose core particle. Protein A/G combines four IgG binding domains from Protein A and two binding domains from Protein G, making it a more general and convenient tool for purifying immunoglobulins. The density of protein A/G on the magnetic agarose bead results in binding capacity similar to or better than traditional agarose resins with the added feature of magnetic handling.

Table 1 Characteristics of the Thermo Scientific™ Pierce™ Protein A/G Magnetic Agarose Beads.

Composition	Recombinant Protein A/G covalently attached to magnetic, highly crosslinked agarose supports
Magnetization	Ferrimagnetic with low remanence
Bead size	10-40 µm
Bead concentration	25% slurry in phosphate buffered saline, 0.01% Tween™-20 Detergent, 0.05% sodium azide
Binding capacity	>40 mg rabbit IgG/mL settled beads

Contents

Contents	Cat. No. 78609	Cat. No. 78610	Storage
Pierce™ Protein A/G Magnetic Agarose Beads	4 mL, supplied at 25% v/v suspension in phosphate buffered saline and 0.01% Tween™-20 Detergent with 0.05% sodium azide	20 mL, supplied at 25% v/v suspension in phosphate buffered saline and 0.01% Tween™-20 Detergent with 0.05% sodium azide	Store at 4°C.

Additional information

- Optimal time for low-pH elution is 10 minutes; exceeding 10 minutes may result in aggregation of antibody and yield reduction.
- Protein A/G has a broader binding range than either Protein A or Protein G individually. Protein A/G binds to all human IgG subclasses; binds somewhat to IgA, IgE, IgM and, to a lesser extent, IgD. Protein A/G is effective for mouse monoclonal antibody purification from IgG subclasses because Protein A/G binds all mouse IgG subclasses but does not bind murine IgA, IgM or serum albumin. For more information, see thermofisher.com for Tech Tip #34: Binding Characteristics for Immunoglobulins and Protein L, A, G and A/G.

Manual purification of antibodies

Materials required but not supplied for manual purification

- 1.5 mL low protein binding microcentrifuge tubes (Product No. 90410)
- Serum, concentrated cell culture supernatant or ascites sample

  Note: Samples can be concentrated using Thermo Scientific™ Pierce™ Protein Concentrators (thermofisher.com/concentrators).
- Magnetic stand (e.g., Thermo Scientific™ DynaMag™–2 magnet; Product No. 12321D)
- Nutating mixer (e.g., Thermo Scientific™ HulaMixer™ Sample Mixer; Product No. 15920D)
- **Binding/Wash Buffer: Phosphate buffered saline; 10 mM phosphate buffer (pH 7.4) with 150 mM NaCl**
- **Elution Buffer: IgG Elution Buffer, pH 2.0 (Product No. 21028) or 0.1 M glycine, pH 2.0-3.0**
- **Neutralization Buffer: High ionic strength alkaline buffer (e.g., 1 M phosphate or 1 M Tris; pH 7.5)**

Perform manual purification of antibodies

- To ensure homogeneity, mix the beads thoroughly before use by repeated inversion, gentle vortexing or using a rotating platform.
- Minimum bead slurry volume recommended for antibody purification is 40 µL.
- The below protocol is designed to purify 200 µg of antibody from 50 µL of serum or 0.5 mL of cell culture supernatant (depending on protein expression). Adjust volumes accordingly based on antibody sample to be purified.

Binding capacity	Settled bead volume
1 mg	25 µL
4 mg	100 µL
10 mg	250 µL
20 mg	500 µL
40 mg	1000 µL

1. Place 40 µL slurry (10 µL settled beads) of Pierce Protein A/G Magnetic Agarose beads into a 1.5mL microcentrifuge tube. Add 460 µL of Binding/Wash buffer to the beads and gently vortex to mix.

   Note: In general, use 10 µL of settled beads per 200 µg of antibody to be purified.
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 0.5 mL of Binding/Wash Buffer to the tube. Invert the tube several times or gently vortex to mix for 1 minute. Collect beads with magnetic stand, then remove and discard the supernatant.
4. Dilute 50 µL of serum or ascites with 450 µL Binding/Wash Buffer. For cell culture supernatant, add up to 500 µL of media depending on antibody expression level.

   Note: Sample volume can be modified according to user preference. If the sample volume is <500 µL, dilute it to a final volume of 500 µL with Binding/Wash Buffer.
5. Add the diluted sample to the tube containing pre-washed magnetic beads and gently vortex or invert to mix.

   Note: Reserve a small volume of load for subsequent SDS-PAGE analysis before adding samples.
6. Incubate the samples at room temperature with mixing for 1 hour.
7. Collect the beads with a magnetic stand, then remove the supernatant. The flowthrough fraction can be saved for subsequent downstream analysis.
8. Add 500 µL of Binding/Wash Buffer to the tube, mix well, collect the beads with a magnetic stand, and remove the supernatant. Repeat this wash once for a total of two washes.
9. Add 500 µL of deionized water to the tube, mix well, collect the beads with a magnetic stand, and remove the supernatant.
10. Add 100 µL of Elution Buffer to the tube, mix well, and incubate 10 minutes at room temperature with occasional mixing.

    Note: Add 100 µL of Elution Buffer per 10 µL of settled beads, e.g. 200 µL for 20 µL settled beads (80 µL slurry).
11. Collect the beads with a magnetic stand and then remove and save the supernatant that contains the eluted antibody. To neutralize the low pH, add 25 µL of Neutralization Buffer for each 100 µL of eluate.

Automated purification of antibodies

Materials required but not supplied for automated purification

- KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head (Product No. 5400630)
- KingFisher™ Deepwell 96 Plate, V-bottom, polypropylene (100-1000 µL; Product No. 95040450)
- 96 Deep-Well Tip Combs for KingFisher™ Flex Magnetic Particle Processor (Product No. 97002534)
- **Binding/Wash Buffer: Phosphate buffered saline; 10mM phosphate buffer (pH 7.4) with 150 mM NaCl**
- **Elution Buffer: IgG elution buffer, pH 2.0 (Product No. 21028) or 0.1 M glycine, pH 2.0-3.0**
- **Neutralization Buffer: High ionic strength alkaline buffer (e.g., 1 M phosphate or 1 M Tris; pH 7.5)**

Prepare instrument and set up plates

Note: The following protocol is designed for use with the KingFisher™ Flex Magnetic Particle Processor. The protocol can be modified according to customer needs using the Thermo Scientific™ BindIt™ Software provided with the instrument.

1. Download the appropriate BindIt™ Software protocol from the product page (Product No. 78609, 78610) on the Thermo Fisher Scientific website 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 Plate set-up.

Plate #	Plate name	Content	Volume
1	Beads	Beads	40 µL
		Binding/Wash Buffer	460 µL
2	Bead wash	Binding/Wash Buffer	500 µL
3	Bind	Sample	50-500 µL
		Binding/Wash Buffer	0-450 µL
4	Wash 1	Binding/Wash Buffer	500 µL
5	Wash 2	Binding/Wash Buffer	500 µL
6	Wash 3	Water	500 µL
7	Elution	Elution Buffer	100 µL
8	Tip plate	KingFisher™ 96 tip comb for DW magnets	—

Note:

- 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.
- Sample volume can be modified according to user preference. If the sample volume is <500 µL, dilute to a final volume of 500 µL with Binding/Wash Buffer.

Perform antibody purification protocol on the Kingfisher Flex instrument

1. Select the protocol using the arrow keys on the instrument keypad and press **Start**. See the KingFisher™ Flex Magnetic Particle Processor Instrument user manual for detailed information.
2. Slide open the door of the instrument's protective cover.
3. Load plates into the instrument according to the protocol requests, placing each plate in the same orientation. Confirm each action by pressing **Start**.
4. After sample processing, remove the plates as instructed by the instrument's display. Press **Start** after each plate. Stop after removing all of the plates.
5. Upon completion, neutralize the low pH by adding 25 µL of Neutralization Buffer for each 100 µL of elution buffer.

Frequently asked questions for the Kingfisher instruments

Question	Answer
Which plates are compatible with the KingFisher™ Flex Magnetic Particle Processor?	The KingFisher™ Flex Magnetic Particle Processor is compatible with the KingFisher™ Flex 24 Deep-Well Plates, KingFisher™ Deepwell 96 Plate, V-bottom, polypropylene, 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 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 Magnetic Particle Processor can be heated using specially designed, interchangeable heating blocks.
Why do the beads stick to the plastic tips and wells? Why does the eluted protein stick to the wells?	Eluted proteins and proteins conjugated to beads can nonspecifically bind to plastics. Adding detergent to Binding/Wash Buffer prevents the protein conjugated to the bead from sticking (0.05%-0.1% Tween™-20 Detergent). Also include a small amount of detergent in the elution buffer 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 products

Product	Cat. no.
Pierce™ Protein A/G Plus Agarose	20424
Pierce™ Protein A Agarose	20334
Pierce™ Recombinant Protein A Agarose	20366
POROS™ MabCapture™ A/G Select	82085
Pierce™ Gentle Ag/Ab Elution Buffer, pH 6.6	21027
Pierce™ Gentle Ag/Ab Binding and Elution Buffer Kit	21030

Limited product warranty

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Troubleshooting

Observation	Possible cause	Recommended action
Minimal protein recovered.	Protein degraded.	Add protease inhibitors.
	Insufficient magnetic beads.	Increase amount of beads used for capture.
	Sample had insufficient amount of target protein.	Increase amount of antibody sample.
Protein does not elute.	Elution conditions were too mild.	Increase incubation time with elution buffer or use more stringent elution buffer.
Multiple nonspecific bands.	Nonspecific protein bound to the magnetic beads.	Increase NaCl in the Binding/Wash Buffer; add non-ionic detergent.
Purified antibody inactive.	Elution conditions were too stringent.	Use a milder elution buffer (e.g., Gentle Elution Buffer; Product No. 21034).
Magnetic beads aggregated.	Magnetic beads were frozen or centrifuged.	Handle beads as directed in instructions.
	Buffer was incompatible with magnetic beads.	Handle beads as directed in instructions.

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Revision history: Pub. No. MAN0015959

Revision	Date	Description
A.0	08 July 2016	New manual

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