invitrogen PRODUCT INFORMATION SHEET

Pierce™ Anti-DYKDDDDK Magnetic Agarose

Catalog Numbers A36797 and A36798

Doc. Part No. 2162708 **Pub. No.** MAN0017395 **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™ Anti-DYKDDDDK Magnetic Agarose provides a fast, convenient method for purification and immunoprecipitation (IP) of DYKDDDDK-tagged proteins from human *in vitro* protein expression systems, bacterial, yeast, and mammalian cell lysates. The amino acid sequence DYKDDDDK, commonly known as the "Flag™ epitope group", is recognized by a high-affinity rat monoclonal antibody (clone L5) that is covalently attached to a magnetite-embedded agarose core particle (Table 1). For protein purification, the magnetic agarose is added to a sample containing DYKDDDDK-tagged protein(s) with the tag on either the N- or the C-terminus. Captured proteins are then magnetically separated from the supernatant, and non-specifically bound proteins can be washed away before dissociating bound DYKDDDDK- tagged protein(s) with elution buffer. The magnetic agarose is removed from the solution using a magnetic stand or an instrument such as the Thermo Scientific™ KingFisher™ Flex Magnetic Particle Processor. Automated instruments are especially useful for higher throughput purifications and screening of purification conditions.

Table 1 Characteristics of the Thermo Scientific™ Pierce™ Anti-DYKDDDDK Magnetic Agarose.

Composition	Anti-DYKDDDDK antibody covalently attached to a magnetic, highly crosslinked agarose support
Magnetization	Ferrimagnetic with low remanence
Bead size	10-40 µm
Operating Temperature	2-30ºC; do not freeze
Bead concentration	25% slurry in phosphate buffered saline, 0.01% Tween™-20 Detergent, 0.02% sodium azide, pH 7.2
Binding capacity	≥3.2 mg DYKDDDDK-tGFP-His protein (~32kDa)/mL settled beads

Contents

Contents	Cat. No. A36797	Cat. No. A36798	Storage
Pierce™ Anti-DYKDDDDK Magnetic Agarose	4 mL, supplied at 25% v/v suspension in phosphate buffered saline and 0.01% Tween™-20 Detergent with 0.02% sodium azide, pH 7.2	20 mL, supplied at 25% v/v suspension in phosphate buffered saline and 0.01% Tween™-20 Detergent with 0.02% sodium azide, pH 7.2	Store at 4°C.

Additional information

- The optimal pH of low-pH elution buffer is 2.8. Use of a lower pH elution buffer (e.g., pH 2.0) will damage DYKDDDDK antibody function.
- Optimal time for low-pH elution is 5 minutes; a second elution is recommended for high abundant target proteins for maximum recovery.
- Magnetic agarose should be maintained in suspension throughout all incubations for efficient processing. Use of a rotator, end-over-end mixer, or ThermoMixer™ device is recommended for manual applications.
- To prevent antibody leach from the magnetic agarose, do not incubate the beads for more than 15 minutes with low-pH elution buffer. Alternatively, perform a competitive elution with 1.5 mg/mL Thermo Scientific™ Pierce™ 3x DYKDDDDK peptide (Product No. A36805)
- The magnetic agarose can be regenerated at least twice using the protocol provided below.
- 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 will vary depending on the size of the DYKDDDDK-tagged protein.
- Both N-terminal and C-terminal DYKDDDDK are recognized by the Pierce™ Anti-DYKDDDDK Magnetic Agarose.
- Protein samples purified using Pierce™ Anti-DYKDDDDK Magnetic Agarose are compatible with immunoprecipitation and western blot analysis.
- Do not use cell lysate containing dithiothreitol (DTT). DTT may denature the DYKDDDDK antibody and cause it to leach from the beads.

Manual purification or immunoprecipitation of DYKDDDDK-tagged protein

Materials required but not supplied for manual purification

- 1.5 mL microcentrifuge tubes
- Sample containing DYKDDDDK-tagged protein
- Magnetic stand (e.g., Thermo Scientific™ DynaMag™-2 Magnet; Product No. 12321D)
- End-over-end mixer or ThermoMixer™ device
- **Binding Buffer: Buffer used to prepare cell lysate (e.g., Thermo Scientific™ B-PER™ Bacterial Protein Extraction Reagent (Product No. 78243) for bacterial cells or Thermo Scientific™ Pierce™ IP Lysis Buffer (Product No. 87788) for mammalian cells**
- **Wash Buffer: Phosphate buffered saline (PBS, Product No. 28372) or 10-100 mM phosphate buffer (pH 7.2) with 150 mM NaCl**
- **Elution Buffer options:**
  - **IgG Elution Buffer, pH 2.8 (Product No. 21004) or 0.1 M glycine, pH 2.8**
  - **Pierce™ 3x DYKDDDDK Peptide (Product No. 36805), 1.5 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: 1 M Tris; pH 8.5 (for use with acid elution)**

Perform manual purification or immunoprecipitation of DYKDDDDK-tagged protein

- Equilibrate magnetic agarose to room temperature before use.
- To ensure homogeneity, mix the magnetic agarose thoroughly before use by repeated inversion, gentle vortexing or using a rotating platform.
- Minimum magnetic agarose slurry volume recommended for immunoprecipitation is 50 µL.
- The amount of lysate needed and incubation times are dependent on the expression level of the DYKDDDDK-tagged protein and require optimization for each specific system. A longer incubation may be required for low abundant target proteins in an IP.
- Up to 200 µL of magnetic agarose slurry can be used with the protocol below. Scale the protocol below for larger volumes.

1. Place 50 µL slurry (12.5 µL settled magnetic agarose) of Pierce™ DYKDDDDK Magnetic Agarose into a 1.5 mL microcentrifuge tube. Add 450 µL of Binding Buffer to the magnetic agarose and gently vortex to mix.
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 500 µL of Binding 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.
4. Add the sample containing DYKDDDDK-tagged protein to the pre-washed magnetic agarose and gently vortex or invert to mix.
   Note: Sample volume can be modified according to user preference. If the sample volume is <300 µL, dilute it to a final volume of 300 µL with Binding Buffer to ensure effective mixing.
5. Incubate the samples at room temperature with mixing for 20 minutes.
6. Collect the beads with a magnetic stand, then remove the supernatant. The flow-through fraction can be saved for subsequent downstream analysis.
7. Add 500 µL of Wash Buffer to the tube, mix well, collect the magnetic agarose with a magnetic stand, and remove the supernatant. Repeat this wash once for a total of two washes.
8. Add 500 µL of purified water to the tube, mix well, collect the beads with a magnetic stand, and remove the supernatant.

Elute DYKDDDDK-tagged protein

Note: Select one of the elution protocols below. If the eluted DYKDDDDK-tagged protein will be used for functional applications or is sensitive to low pH, then elute the protein with Pierce™ 3x DYKDDDDK Peptide.

Gentle Elution Protocol

1. Prepare Pierce 3x DYKDDDDK Peptide at 1.5 mg/mL in PBS.
2. Add 100µL of 1.5mg/mL Pierce 3x DYKDDDDK Peptide to the tube, mix well, and incubate 10 minutes at room temperature with frequent vortexing or in a ThermoMixer™ device at 1400 rpm.
3. Collect the beads with a magnetic stand and then remove and save the supernatant that contains the eluted target.
4. Repeat elution step once for maximum recovery (this is recommended for complete recovery of high abundant targets).

Note: Pierce™ 3x DYKDDDDK Peptide may interfere with protein determination assays and absorbance at 280nm. Peptide can be removed by desalting (e.g., Zeba™ Spin Desalting Columns, Product No. 89891).

Acid Elution Protocol

1. Add 100 µL of Elution Buffer, pH 2.8 to the tube, mix well, and incubate 5 minutes at room temperature with frequent vortexing or in a ThermoMixer™ device at 1400 rpm.
2. Collect the magnetic agarose with a magnetic stand and then remove and save the supernatant that contains the eluted target. To neutralize the low pH, add 15 µL of Neutralization Buffer for each 100 µL of eluate.
3. Repeat elution step once for maximum recovery (this is recommended for complete recovery of high abundant targets).
   Note: Following acid treatment, immediately transfer the magnetic agarose into PBS to prevent denaturation of DYKDDDDK antibody.

Sample Buffer Protocol

1. Add 100µL of SDS-PAGE Sample Buffer to the tube.
2. Gently vortex to mix and incubate the sample at 95-100ºC for 5-10 minutes.
3. Collect the magnetic agarose with a magnetic stand and then remove and save the supernatant that contains the eluted target.
   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 buffer.
   Note: Do not re-use magnetic agarose after treatment with sample buffer.

Regenerate magnetic agarose

Note: The magnetic agarose can be regenerated up to 2 times.
Note: A minimum volume of 100µL of magnetic agarose slurry is recommended.

1. Add 10 bead volumes of PBS to collected magnetic agarose (e.g., add 250 µL PBS to 25 µL of settled magnetic agarose). Mix well, collect the magnetic agarose with a magnetic stand and remove the supernatant. Repeat this wash once for a total of two washes.
2. Add 10 bead volumes of deionized water to collected magnetic agarose. Mix well, collect the magnetic agarose with a magnetic stand and remove the supernatant.
3. Add 5 bead volumes of Elution Buffer to tube, mix well, and incubate 5 minutes at room temperature with frequent vortexing or in a ThermoMixer™ device at 1400 rpm.
4. Collect the magnetic agarose with a magnetic stand and remove the supernatant. Repeat this acid wash twice for a total of three washes.
5. Add 10 bead volumes of PBS to collected magnetic agarose. Mix well, collect the magnetic agarose with a magnetic stand and remove the supernatant. Repeat this wash once for a total of two washes.
6. Store magnetic agarose in PBS at 25% slurry containing 0.01% Tween™-20 and 0.02% sodium azide at 4ºC.

Automated purification or immunoprecipitation of DYKDDDDK-tagged protein

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 Buffer: Buffer used to prepare cell lysate (e.g., Thermo Scientific™ B-PER™ Bacterial Protein Extraction Reagent (Product No. 78243) for bacterial cells or Thermo Scientific™ Pierce™ IP Lysis Buffer (Product No. 87788) for mammalian cells
- Wash Buffer: Phosphate buffered saline (PBS, Product No. 28372) or 10-100 mM phosphate buffer (pH 7.2) with 150 mM NaCl
- Elution Buffer options:

  IgG Elution Buffer, pH 2.8 (Product No. 21004) or 0.1 M glycine, pH 2.8
  Pierce™ 3x DYKDDDDK Peptide (Product No. 36805), 1.5 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: 1 M Tris; pH 8.5 (for use with acid elution)

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. A36797, A36798) 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	50µL slurry
		Binding Buffer	450 µL
2	Bead pre-wash	Binding Buffer	500 µL
3	Bind	Sample	500 µL
4	Wash 1	Wash Buffer	500 µL
5	Wash 2	Wash Buffer	500 µL
6	Wash 3	Deionized Water	500 µL
7	Elution	Acid Elution Buffer or 1.5mg/mL Pierce™3x DYKDDDDK Peptide	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 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 Buffer. If the sample volume is higher than 500 µL, adjust the volume in the BindIt™ protocol.
- Bead volume can be modified according to user preference. Adjust the Binding Buffer volume to allow for a final volume of 500 µL in the wells containing beads.

Perform purification/IP 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
4. Start.
5. 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.
6. Upon completion, neutralize the low pH by adding 15 µL of Neutralization Buffer for each 100 µL of Acid 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.
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™ Anti-DYKDDDDK Affinity Resin	A36801
Pierce™ Anti-HA Magnetic Beads	88836
Pierce™ Anti-HA Agarose	26181
Pierce™ Anti-c-Myc Magnetic Beads	88842
Pierce™ Anti-c-Myc Agarose	20168

Troubleshooting

Observation	Possible cause	Recommended action
Minimal protein recovered.	Protein degraded.	Add protease inhibitors.
	Insufficient magnetic agarose.	Increase amount of beads used for capture.
	Sample had insufficient amount of target protein.	Increase amount of sample.
Protein does not elute.	Elution conditions were too mild.	Perform a second elution or increase incubation time with elution buffer. Do not exceed 15 minutes if magnetic agarose is to be reused.
		Use more stringent elution buffer (for acid elution, use 0.1M Glycine, pH 2.0). Magnetic agarose cannot be reused after treatment with 0.1M Glycine, pH 2.0.
Multiple nonspecific bands.	Nonspecific protein bound to the magnetic agarose.	Increase NaCl in the Wash Buffer; add non-ionic detergent (e.g., 0.025% Tween™-20).
Purified DYKDDDDK-protein is inactive.	Elution conditions were too stringent.	Elute with 3x DYKDDDDK peptide (Product No. A36805).
DYKDDDDK-tagged protein is in the flow-through.	Magnetic agarose was overloaded.	Reduce amount of sample or increase the amount of magnetic agarose.
	Magnetic agarose was not regenerated after use.	Regenerate magnetic agarose using protocol provided in the manual.
	DYKDDDDK tag was not accessible to magnetic agarose.	Switch tag to the other terminus of the protein.
		Add a linker (i.e., a spacer) between the tag and the protein.
Magnetic agarose aggregated.	Magnetic agarose was frozen or centrifuged.	Handle beads as directed in instructions.
	Buffer was incompatible with magnetic agarose.	Handle beads as directed in instructions.
Eluted DYKDDDDK-tagged protein is functionally inactive.	Protein was sensitive to low pH elution.	Use 3x DYKDDDDK peptide for competitive elution. Use Enterokinase (Cat No. E18001) to cleave N-ter DYKDDDDK-tag to release protein of interest. To remove Enterokinase from the reaction, use EK-Away Resin (Cat No. R18001).

Manufacturer: Pierce Biotechnology, Inc. | Thermo Fisher Scientific | 3747 N. Meridian Road | Rockford, Illinois 61101 USA