Pierce™ Anti-DYKDDDDK Affinity Resin

Catalog Numbers A36801, A36803, A36804

Doc. Part No. 2162707 **Pub. No.** MAN0017398 **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 Affinity Resin provides a fast, convenient method for purification and immunoprecipitation (IP) of DYKDDDDK-tagged proteins from in vitro protein expression systems, bacteria, 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 Ultralink™ Biosupport (Table 1). For protein purification, the resin is added to a sample containing DYKDDDDK-tagged protein(s) with the tag on either the N- or the C-terminus. Following capture of DYKDDDDK-tagged proteins, non-specifically bound proteins can be washed away before dissociating bound target protein(s) with elution buffer. The Pierce™ Anti-DYKDDDDK Affinity Resin is recommended for use in spin purification columns or cartridges for FPLC instruments.

UltraLink™ Biosupport is a rigid, hydrophilic, highly crosslinked, copolymeric and porous resin with high coupling capacity. The porosity, rigidity and durability of the resin also make it suitable for medium-pressure, fast-flow techniques involving large sample volumes.

Table 1 Characteristics of the Thermo Scientific™ Pierce™ Anti-DYKDDDDK Affinity Resin.

Composition	Anti-DYKDDDDK antibody covalently attached to an Ultralink™ Biosupport
Particle Size	50-80 µm
Recommended Linear Velocity	≤150 cm/hr
Operating Temperature	2-30ºC; do not freeze
Particle Concentration	50% slurry in phosphate buffered saline, 0.02% sodium azide, pH 7.5
Binding capacity	≥3.0 mg DYKDDDDK-tGFP-His protein (~32 kDa)/mL settled resin

Contents

Contents			Storage
Pierce™ Anti-DYKDDDDK Affinity Resin	**Cat. No. A36801**	2 mL, supplied at 50% v/v suspension in phosphate buffered saline with 0.02% sodium azide, pH 7.5	Store at 4°C.
	Cat. No. A36803	10 mL, supplied at 50% v/v suspension in phosphate buffered saline with 0.02% sodium azide, pH 7.5	
	Cat. No. A36803	100 mL, supplied at 50% v/v suspension in phosphate buffered saline with 0.02% sodium azide, pH 7.5	

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 and prevent reuse of resin.
- UltraLink™ Resin should be maintained in suspension throughout all incubations for efficient processing. Use of a rotator or end-over-end mixer is recommended.
- For gentle elution, perform a competitive elution with 1.5mg/ml Thermo Scientific™ Pierce™ 3x DYKDDDDK peptide (Product No. A36805)
- The UltraLink™ Resin can be regenerated at least four times 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 Affinity Resin.
- Protein samples purified using Pierce™ Anti-DYKDDDDK Affinity Resin 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 resin.

Immunoprecipitation of DYKDDDDK-tagged protein

Materials required but not supplied for immunoprecipitation with spin columns

- Spin columns and collection tubes (e.g., Thermo Scientific™ Pierce™ Spin Columns, 1mL, Product No. 69725)
- Sample containing DYKDDDDK-tagged protein
- End-over-end mixer
- **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 immunoprecipitation of DYKDDDDK-tagged protein

- Equilibrate resin to room temperature before use.
- To ensure homogeneity, mix the Pierce™ DYKDDDDK Affinity Resin thoroughly before use by repeated inversion or using a rotating platform.
- Minimum resin slurry volume recommended for immunoprecipitation is 50 µL (25 µL settled resin).
- The amount of lysate needed and incubation times are dependent on the expression level of the DYKDDDDK-tagged protein and required optimization for each specific system. A longer incubation may be required for low abundant target proteins in an IP.
- Higher volumes of resin can be used by scaling the protocol below.
- Use the same 2 mL collection tube for all of the pre-wash steps. Use clean 2 mL collection tubes for collecting flow-through and elution.
- Acid elution is as efficient as peptide elution using the protocols below.

1. Add 200 µL of Binding Buffer to a 1 mL spin column containing a plug on the tip.
2. Using a wide-bore pipette tip, place 50 µL slurry (25 µL settled resin) of Pierce™ DYKDDDDK Affinity Resin into the column. Remove plug and insert spin column into a 2 mL collection tube.
3. Cap the column and centrifuge the column assembly for 1 minute at 1,000 × *g*. Discard the liquid from the collection tube.
4. Plug the column tip and add 250 µL (10 bed volumes) Binding Buffer. Cap the column and flick to resuspend the resin. Remove plug and insert column into collection tube. Centrifuge assembly for 1 minute at 1,000 × *g*. Repeat wash once for a total of two washes.
5. Plug the column tip and add prepared sample to resin (300-800 µL). Cap the column and resuspend the resin by flicking or gently vortexing.
   Note: If sample volume is <200 µL, add Binding Buffer to at least 300 µL to ensure adequate mixing.
6. Place column on a rotator and mix for 20 minutes at room temperature.
   Note: Incubation can be performed at 4°C, but a longer incubation (at least 1 hour) is recommended.

7. Remove plug from column and insert into clean collection tube. Centrifuge for 1 minute at 1,000 × *g*. The flow-through fraction can be saved for subsequent downstream analysis.
8. Plug the column tip and add 250 µL (10 bed volumes) Wash Buffer. Cap the column and flick to resuspend the resin. Remove plug and insert column into collection tube. Centrifuge assembly for 1 minute at 1,000 × *g*. Repeat wash once for a total of two washes.
9. Plug the column tip and add 250 µL (10 bed volumes) purified water. Cap the column and flick to resuspend the resin. Remove plug and insert column into collection tube. Centrifuge assembly for 1 minute at 1,000 x *g*.

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™ 3× DYKDDDDK Peptide.

Gentle Elution Protocol

1. Prepare Pierce 3x DYKDDDDK peptide at 1.5 mg/mL in PBS.
2. Plug the column tip and add 200µL of 1.5 mg/mL Pierce 3x DYKDDDDK peptide. Cap the column and gently vortex to resuspend the resin.
3. Place the column on a rotator and mix for 5 minutes at room temperature.
   Note: Vortex 2-3 times during the incubation to ensure the slurry is properly mixing.
4. Remove plug from column and insert into clean collection tube. Centrifuge for 1 minute at 1,000 × g. Save the eluate that contains the eluted target.
5. Repeat elution step once for maximum recovery (this is recommended for complete recovery of high abundant targets).

Acid Elution Protocol

1. Plug the column tip and add 200µL of Elution Buffer, pH 2.8. Cap the column and vortex for 3 seconds to resuspend the resin. Remove the plug and insert column into a clean collection tube. Centrifuge for 1 minute at 1,000 × *g*.
2. To neutralize the low pH, add 30 µL of Neutralization Buffer for each 200 µL of eluate.
3. Repeat elution step once for maximum recovery (this is recommended for complete recovery of high abundant targets).
   Note: Immediately following acid treatment, immediately transfer the resin into PBS to prevent denaturation of DYKDDDDK antibody.

Sample Buffer Protocol

1. Transfer resin to a tube.
2. Add 100µL of SDS-PAGE Sample Buffer to the tube.
3. Gently vortex to mix and incubate the sample at 95-100°C for 5-10 minutes.
4. Centrifuge the resin 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 2.5 µL of 2 M DTT to the 100 µL sample buffer.
   Note: Do not re-use resin after treatment with sample buffer.

Regenerate resin

Note: The Pierce™ DYKDDDDK Affinity Resin can be regenerated up to 7 times.
Note: A minimum volume of 100 µL of resin slurry is recommended.

1. Wash resin with 20 bed volumes of PBS.
2. Wash resin with 10 bed volumes of purified water.
3. Wash resin with 3 × 5 bed volumes of Elution Buffer.
4. Wash resin with 20 bed volumes of PBS.
5. Store resin in PBS at 50% slurry containing 0.02% sodium azide at 4°C.

Purification of DYKDDDDK-tagged protein

Materials required but not supplied for purification with spin columns

- Spin columns (e.g., Thermo Scientific™ Pierce™ Centrifuge Columns, 5mL, Product No. 89897, or 10mL, Product No. 89898)
- 15mL conical tubes
- Sample containing DYKDDDDK-tagged protein
- End-over-end mixer or rotator
- **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**
- **Neutralization Buffer: 1 M Tris; pH 8.5**

Perform purification of DYKDDDDK-tagged protein using a spin column

- Equilibrate resin to room temperature before use.
- To ensure homogeneity, mix the Pierce™ DYKDDDDK Affinity Resin thoroughly before use by repeated inversion or using a rotating platform.
- The following protocol uses 2mL of resin slurry (1mL settled resin). The protocol can be scaled for different volumes of resin (e.g., double wash volumes for 2mL settled resin).
- 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.
- Use the same 15mL collection tube for all of the pre-wash steps. Use clean 15mL collection tubes for collecting flow-through and elution.
- Acid elution is as efficient as peptide elution using the protocols below.

1. Add 1 mL of Binding Buffer to a 5 mL centrifuge column.
2. Using a wide-bore pipette tip, place 2 mL of Pierce™ DYKDDDDK Affinity Resin (1 mL settled resin) into the column. Twist off bottom closure and insert column into a 15 mL collection tube.
3. Cap column and centrifuge assembly for 2 minutes at 1,000 × *g*. Discard the liquid from the collection tube.
4. Plug the column tip and add 5 mL (5 bed volumes) Binding Buffer. Cap the column and mix end over end to resuspend the resin. Remove plug and insert column into collection tube. Centrifuge assembly for 2 minutes at 1,000 × *g*. Repeat wash once for a total of two washes.
5. Plug the column tip and add prepared sample to resin. Cap the column and resuspend the resin by mixing end over end.
6. **Note:** If sample volume is < 2 mL, add Binding Buffer to at least 2 mL to ensure adequate mixing.
7. Place column on a rotator and mix for 20 minutes at room temperature.
8. **Note:** Incubation can be performed at 4°C, but a longer incubation (at least 1 hour) is recommended.
9. Remove plug from column and insert into clean collection tube. Centrifuge for 2 minutes at 1,000 × *g*. The flow-through fraction can be saved for subsequent downstream analysis.
10. Plug the column tip and add 5 mL (5 bed volumes) Wash Buffer. Cap the column and mix end over end. Remove plug and insert column into collection tube. Centrifuge assembly for 2 minutes at 1,000 × *g*. Repeat wash twice for a total of three washes.
11. Plug the column tip and add 5 mL (5 bed volumes) purified water. Cap the column and mix end over end. Remove plug and insert column into collection tube. Centrifuge assembly for 2 minutes at 1,000 × *g*.

Elution of 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. Plug the column tip and add 2 mL of 1.5 mg/mL Pierce™ 3x DYKDDDDK peptide. Cap the column and resuspend resin by mixing end over end.
3. Place the column on a rotator and mix for 5 minutes at room temperature.
4. Remove plug from column and insert into clean collection tube. Centrifuge for 2 minutes at 1,000 × *g*. Save the eluate that contains the eluted target.
5. 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 280 nm. Peptide can be removed by desalting (e.g., Zeba™ Spin Desalting Columns, Product No. 89891).

Acid Elution Protocol

1. Plug the column tip and add 2mL Elution Buffer, pH 2.8. Cap the column and vortex for 3 seconds to resuspend the resin. Remove the plug and insert column into a clean collection tube. Centrifuge for 1 minute at 1,000 x g.
2. To neutralize the low pH, add 150 µL of Neutralization Buffer for each 1mL of eluate.
3. Repeat elution step once for maximum recovery (this is recommended for complete recovery of high abundant targets).

Note: Immediately following acid treatment, immediately transfer the resin into PBS to prevent denaturation of DYKDDDDK antibody.

Regenerate resin: See the protocol on page 3.

Materials required but not supplied for purification with a liquid chromatography (LC) system

- Suitable LC System
- Empty Column for resin packing (follow column manufacturer's protocol for packing)
- Sample containing DYKDDDDK-tagged protein (to avoid sample loss, it is recommended to not exceed maximum resin-binding capacity)
- End-over-end mixer or rotator
- **Wash Buffer (degas or filter buffers through a 0.45µm filter before use):**
  - **Phosphate buffered saline (PBS, Product No. 28372) or 10-100 mM phosphate buffer (pH 7.2) with 150 mM NaCl**
  - **Ultrapure water**
- **Elution Buffer options(degas or filter buffers through a 0.45µm filter before use):**
  - **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**

Perform purification of DYKDDDDK-tagged protein using a Liquid Chromatography System

- To ensure homogeneity, mix the Pierce™ DYKDDDDK Affinity Resin thoroughly before use by repeated inversion or using a rotating platform.
- Protein yield and purity are dependent upon the expression level, conformations and solubility characteristics of the recombinant fusion protein, as well as the buffer conditions and flow rates used. Therefore, it is important to optimize these parameters before attempting a large-scale purification. For best results, perform a small-scale test to estimate the expression level and determine the solubility of each DYKDDDDK-tagged protein.
- Decreasing the flow rate during sample load will increase binding capacity.
- To avoid sample loss, try not to exceed the maximum resin-binding capacity for the target protein for the purification conditions used. Volumes will vary based on the protein and level of expression efficiency, and will have to be determined and optimized for each over-expressed protein. Typically over-expressed proteins represent 1-30% of the final sample protein concentration. Adjust resin volumes as appropriate; total available capacity should be 20-40% higher than what is needed.
- Optimization of cell lysis procedures is critical for maximizing protein yield. Some methods for protein extraction include using commercially available detergent-based reagents, such as Thermo Scientific B-PER Bacterial Protein Extraction Reagent with Enzymes (Product No. 90078), and mechanical methods such as sonication or French press. Confirm cell disruption before proceeding to protein purification. Add EDTA-free protease inhibitors, such as Thermo Scientific Halt Protease and Phosphatase Inhibitor Cocktail, EDTA-free (Product No. 78437), during lysis procedures to protect proteins from degradation.
- For liquid chromatography (LC) applications, use highly pure buffer components and water. Degas or filter buffers through a 0.45µm filter before use.

1. Pack an appropriate-sized column with resin according to column manufacturer's protocol. Ensure the packing flow rate is at least 20% faster than the flow rate that will be used during purification.
2. Equilibrate the column and all buffers to working temperature. Purifications can be performed at room temperature or at 4°C. Ensure all solutions are degassed.
3. Prepare the LC system by washing pumps and filling tubing with wash buffer. To avoid introducing air into the system, allow a few drops of buffer to flow from the tubing into the column top. Connect column to the tubing.
4. Equilibrate the column with 5-10 column volumes of the Equilibration/Wash Buffer at a flow rate of 300 cm/hr or less.
5. Apply any sample volume that does not exceed column-binding capacity for target protein at a flow rate of 150 cm/hr or less.
   Note: Binding capacity is flow rate- and protein-dependent. Decreasing the flow rate during the sample load will increase binding capacity. Higher flow rates will decrease production time but will result in a small portion of the target protein in the flow-through fraction.
   Note: For maximum binding, prepare sample by mixing protein extract 1:1 with Equilibration/Wash Buffer (to adjust the sample to the ionic strength and pH of the Equilibration/Wash Buffer) before applying to the column.
   Note: If the sample contains insoluble matter, centrifuge or filter (0.45µm filter) before use.
6. Wash the resin at a flow rate of 150 cm/hr or less with 10-15 column volumes of Equilibration/Wash Buffer or until the absorbance approaches baseline.

7. Elute at a flow rate of 150 cm/hr or less with approximately 5-10 column volumes of Elution Buffer and collect fractions.
   Note: Monitor protein elution by measuring the absorbance of the fractions at 280 nm. The eluted protein can be directly analyzed by SDS-PAGE.
8. Regenerate the column by washing with 10 column volumes of IgG Elution Buffer, pH 2.8 (Product No. 21004) or 0.1 M glycine, pH 2.8 at a flow rate of 150 cm/hr or less. The column is now ready for reuse (proceed to step 1 above) or storage (proceed to step 9).
9. For storage, equilibrate the column with 5 column volumes of PBS containing 0.05% sodium azide or 20% ethanol. Seal the column and store at 4°C.

Related products

Product	Cat. no.
Pierce™ Anti-DYKDDDDK Magnetic Agarose	A36797
Pierce™ Anti-HA Agarose	26181
Pierce™ Anti-HA Magnetic Beads	88836
Pierce™ Anti-c-Myc Agarose	20168
Pierce™ Anti-c-Myc Magnetic Beads	88842

Troubleshooting

Observation	Possible cause	Recommended action
Minimal protein recovered.	Protein degraded.	Add protease inhibitors.
	Insufficient resin.	Increase amount of resin used for capture.
	Sample had insufficient amount of target protein.	Increase amount of sample.
Protein does not elute.	Elution conditions were too mild.	Increase incubation time with elution buffer.
		Use more stringent elution buffer (e.g., 0.1M Glycine, pH 2.0). Resin cannot be reused if treated with pH 2.0 acid.
Multiple nonspecific bands.	Nonspecific protein bound to the resin.	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 flowthrough.	Resin was overloaded.	Reduce amount of sample added to the column or increase the amount of resin.
	Resin was not regenerated after use.	Regenerate resin using protocol provided in the manual.
	DYKDDDDK tag was not accessible to resin.	Switch tag to the other terminus of the protein.
		Add a linker (i.e., a spacer) between the tag and the protein.

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