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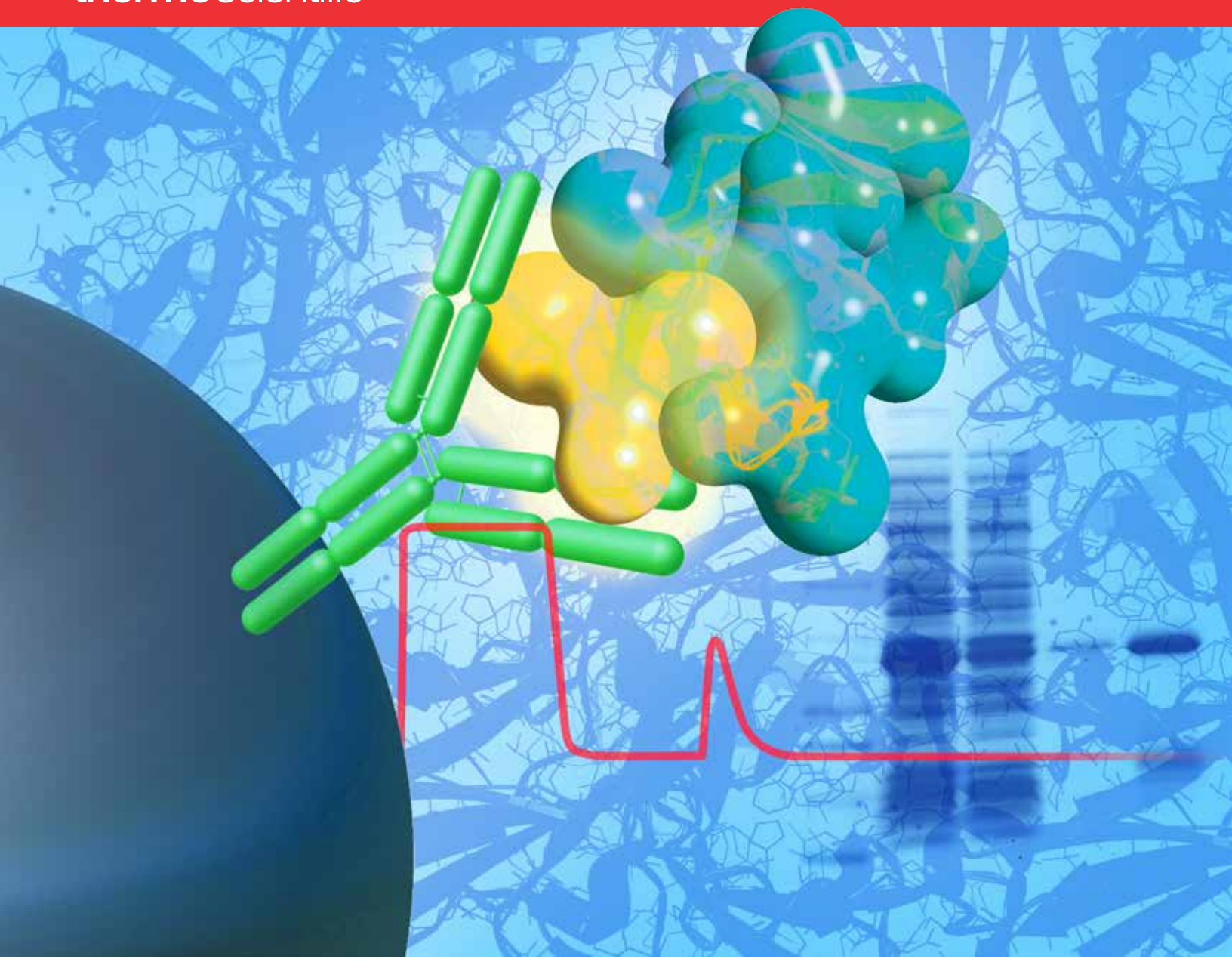


Tools and reagents for recombinant protein purification

ThermoFisher SCIENTIFIC

Introduction

The development of recombinant DNA technology enabled the production of proteins that were originally extracted from tissues and secretions to be produced synthetically with high purity and yield. Multiple-host expression systems including mammalian, insect, bacterial, yeast, algal, and cell-free systems are available to deliver protein at milligram to gram yields, and are easy to transform with a DNA vector containing the gene of interest. Typically, an affinity tag sequence (additional amino acids, a functional domain, or a whole protein) is cloned in frame with the DNA sequence of the target protein, and will flank either the N- or C-terminus, to aid in purification and analysis. These additions are known as fusion tags.

The properties of fusion tags allow tagged proteins to be manipulated easily in the laboratory. Most significantly, the well-characterized tag-ligand chemistry enables single-step affinity purification of tagged molecules using immobilized versions of their corresponding ligands. Antibodies to fusion tags are also widely available for use in downstream detection and assay methods, eliminating the need to obtain or develop a probe for each specific recombinant protein.

Common fusion tags include DYKDDDDK (FLAG™), c-Myc, HA, 6xHis, and glutathione *S*-transferase (GST). The FLAG, c-Myc, and HA sequences are called epitope tags because they require specific antibodies (e.g., immobilized anti-HA antibody) for purification. Epitope tags are more often used for small-scale immunoprecipitation (IP) or co-IP because their gentle elution conditions generally do not disrupt protein interactions. For bench- to pilot-scale applications, 6xHis or GST is the preferred choice of tag, due to the cost-effectiveness of the affinity ligands (Ni-NTA or cobalt for 6xHis and glutathione for GST) used for scale-up.

Contents

Resin selection guide

We offer a variety of Thermo Scientific™ purification resins for the purification and processing of recombinant proteins. Our resins are available in multiple base supports and formats to accommodate a variety of needs, from high-throughput batch screening to batch- and pilot-scale purification (microgram to gram quantities of protein) (Table 1). These include ligands targeting a variety of fusion tags: FLAG epitope, c-Myc, HA, 6xHis, and GST (Table 2).

Table 1. Select your affinity resin based on purification scale and application.

Scale	High-throughput screening	High-throughput batch	Batch	Pilot
Description	Small scale, automation-compatible	Lab or bench scale, automation possible	Lab or bench scale	Scale-up desired
Yield	Microgram	Milligram	Milligram	Gram
Format	Magnetic particle processor	Magnetic particle processor, 96-well spin plate (agarose)	Gravity flow, spin column (agarose), fast protein liquid chromatography (FPLC) at low flow rates	FPLC at medium flow rates
Application	High-throughput screening, interaction studies (IP, co-IP, pull-down), mutational analysis	High-throughput screening interaction studies (IP, co-IP, pull-down), mutational analysis requiring mg scale	Functional assays, structural analysis	Structural analysis, intermediate-scale production
Recommended resin type	Magnetic bead (1–2.8 µm)	Magnetic agarose (10–40 µm) Agarose (45–165 µm)	Agarose (45–165 µm) Superflow (45–165 µm) UltraLink resin (40–70 µm)	Superflow (45–165 µm) UltraLink resin (40–70 µm)

Table 2. Recombinant protein purification selection guide.

Tag	Ligand	Features	Recommended product	High-throughput screening	High-throughput batch	Batch	Pilot
DYKDDDDK (FLAG)	Anti-FLAG	Immobilized antibody	Pierce Anti-DYKDDDDK Magnetic Agarose		✓		
			Pierce Anti-DYKDDDDK Affinity Resin (UltraLink resin)			✓	✓
c-Myc	Anti–c-Myc	Immobilized antibody	Pierce Anti–c-Myc Magnetic Beads	✓			
			Pierce Anti–c-Myc Agarose (Superflow)			✓	✓
HA	Anti-HA	Immobilized antibody	Pierce Anti-HA Magnetic Beads	✓			
			Pierce Anti-HA Agarose			✓	
6xHis	Ni-NTA or Ni-IDA	Higher protein yield	Pierce Ni-NTA Magnetic Agarose Beads		✓		
			ProBond Nickel Chelating Resin			✓	
			HisPur Ni-NTA Agarose Resin			✓	
			HisPur Ni-NTA Superflow Resin				✓
	Cobalt	Higher protein purity	Dynabeads His-Tag Isolation Magnetic Beads	✓			
			HisPur Cobalt Agarose Resin			✓	
			HisPur Cobalt Superflow Resin				✓
GST	Glutathione	Solubility and purification tag	Pierce Glutathione Magnetic Agarose Beads		✓		
			Pierce Glutathione Agarose			✓	
			Pierce Glutathione Superflow				✓

Epitope-tagged protein purification using immunoaffinity ligands

An epitope is the part of an antigen that is recognized by the immune system. Epitope tags are short peptide sequences that are selected because high-affinity antibodies can be readily produced in different species. The gene from the target protein is inserted into the epitope tag vector, and the target protein with its tag is expressed in cells by transfection of the vector. By choosing an epitope for which an antibody is available, the technique makes it possible to purify and detect proteins for which no antibody or affinity ligand is available. Common epitope tags that are immunoaffinity-purified include DYKDDDDK (or FLAG epitope), c-Myc, and HA.

We offer multiple base supports with immobilized antibodies against the DYKDDDDK (DYKD4K), c-Myc, and HA tags for immunoaffinity purification of these epitope-tagged proteins. Our portfolio is designed to meet small-scale (screening) to batch-scale needs (Table 3).

Highlights:

- **Specific**—highly specific monoclonal antibody–based ligands enable high yield and high purity for immunoprecipitation or small- to pilot-scale protein purification
- **Low nonspecific binding**—stable beads and target-specific antibody minimize off-target binding
- **Scalable**—available in larger package sizes for purification scale-up and more economical pricing
- **Versatile**—multiple resin formats available that can be used in spin or gravity columns as well as in FPLC cartridges
- **Automation-friendly**—magnetic agarose or beads recommended for high-throughput applications
- **Convenient**—reagents to elute and detect tagged fusion proteins are available separately

Table 3. Epitope tag immunoaffinity purification selection guide.

	Pierce Anti-DYKDDDDK Magnetic Agarose	Pierce Anti-DYKDDDDK Affinity Resin (UltraLink resin)	Pierce Anti–c-Myc Magnetic Beads	Pierce Anti–c-Myc Agarose (Superflow)	Pierce Anti-HA Magnetic Beads	Pierce Anti-HA Agarose
Bead or resin size	10–40 µm	50–80 µm	1 µm	45–165 µm	1 µm	45–165 µm
Binding capacity	≥3 mg/mL	≥3 mg/mL	≥100 µg/mL	60–150 nmol/mL	≥100 µg/mL	102–144 nmol/mL
Maximum linear flow rate (cm/hr)	NA	150	NA	1,200	NA	700
Support	Magnetite-embedded 6% beaded agarose	Durable, beaded polyacrylamide support	Magnetite-coated polymer	Highly crosslinked 6% beaded agarose	Magnetite-coated polymer	4% beaded agarose
Recommended application scale	High-throughput batch	Batch*, pilot	High-throughput screening	Batch, pilot	High-throughput screening	Batch
Formats available	Loose bead	Loose resin	Loose bead	Loose resin	Loose bead	Loose resin

* UltraLink resin is recommended for spin column or FPLC formats only.

Learn more at **thermofisher.com/epitope-tag-purification**

Pierce Anti-DYKDDDDK Magnetic Agarose and Affinity Resin

Highly specific immobilized antibody supports for epitope-tagged protein purification

The Thermo Scientific™ Pierce™ Anti-DYKDDDDK Magnetic Agarose and Affinity Resin are ideal for the isolation of protein complexes with multiple subunits, because the mild purification process tends not to disrupt these interactions. Additionally, the epitope tag may be used in tandem, commonly the 3x FLAG peptide: DYKDHDG-DYKDHDI-DYKDDDDK, with the final tag encoding an enterokinase cleavage site. This tag can be fused to the C-terminus or the N-terminus of a protein, inserted within a protein, or used in conjunction with other affinity tags, such as 6xHis, HA, or c-Myc.

The FLAG tag (peptide sequence DYKDDDDK, 1,012 Da) is a short, hydrophilic protein tag commonly used in conjunction with antibodies in protein pull-down assays to study protein–protein interactions. Because of its hydrophilic nature, the FLAG tag is commonly found on the surface of a fusion protein, which makes it more available as an epitope for binding to antibodies. In addition, the high hydrophilicity and small size of the FLAG tag tend to interfere less with protein expression, proteolytic maturation, antigenicity, and function. FLAG tags are also easily removed by enterokinase (EK).

The FLAG epitope is recognized by a high-affinity rat monoclonal antibody (clone L5) that is covalently attached to a magnetic agarose or Thermo Scientific™ UltraLink™ resin. The blocked magnetic agarose can be used manually with a magnetic stand, as well as with automated platforms, such as Thermo Scientific™ KingFisher™ instruments, for high-throughput workflows. The magnetic agarose provides a fast, convenient method for purification and immunoprecipitation (IP) of DYKDDDDK-tagged proteins expressed in cell-free, bacterial, yeast, and mammalian expression systems. The affinity resin is composed of a highly crosslinked, rigid, acrylamide-based support (UltraLink resin) that is ideal for scale-up applications and can be used in FPLC applications.

DYKDDDDK-tagged proteins are easily eluted from the magnetic agarose and affinity resin using 0.1 M glycine (pH 2.8). For gentle elution, Thermo Scientific™ Pierce™ 3x DYKDDDDK Peptide is available to competitively elute DYKDDDDK-tagged fusion proteins from both immobilized anti-DYKDDDDK affinity supports.

The results of protein yield, purity, and activity between Pierce Anti-DYKDDDDK supports and other suppliers is shown in Figures 1–5.

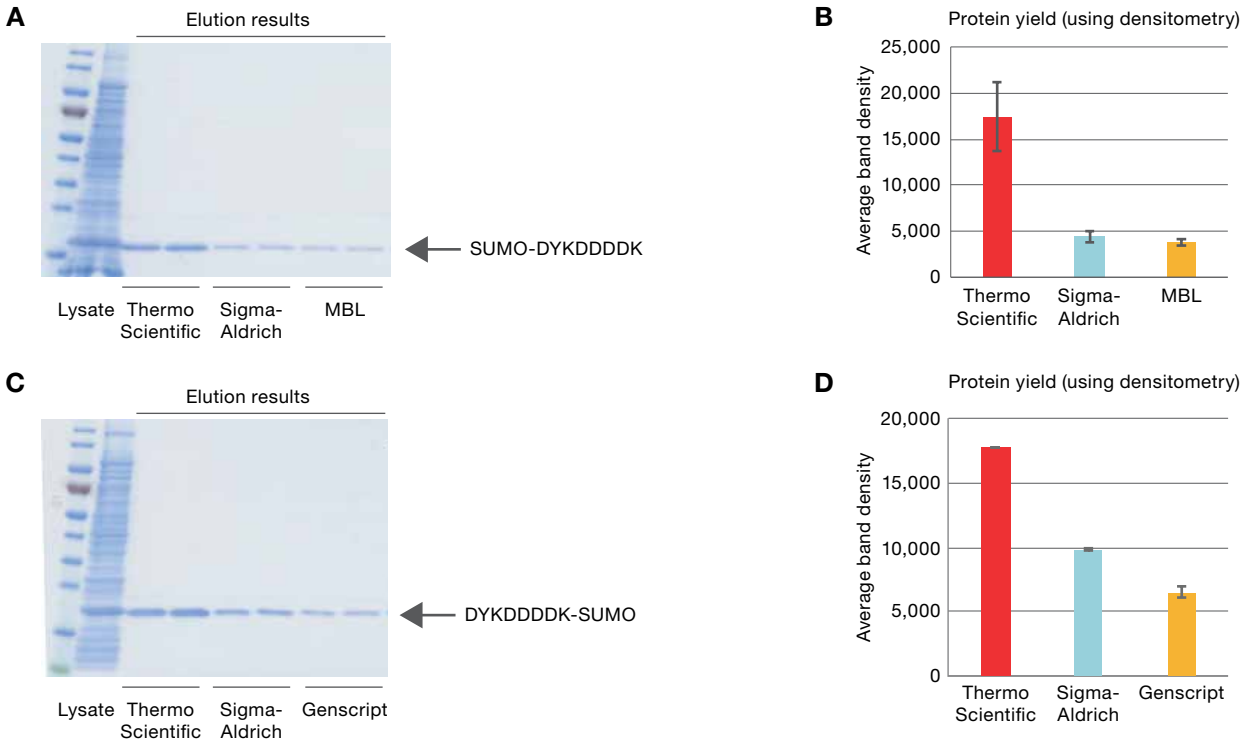


Figure 1. Comparison of DYKDDDDK-tagged SUMO protein purification results using Pierce Anti-DYKDDDDK supports and supports from other suppliers. C- and N-terminal DYKDDDDK-tagged SUMO proteins were expressed in *E. coli* and purified using two Pierce Anti-DYKDDDDK and other suppliers' supports. Tagged protein was competitively eluted with Pierce 3x DYKDDDDK Peptide and analyzed by SDS-PAGE **(A, C)** and densitometry **(B, D)** using the Invitrogen™ iBright™ Imaging System. The results for the Pierce Anti-DYKDDDDK Magnetic Agarose **(A, B)**, Pierce Anti-DYKDDDDK Affinity Resin **(C, D)** Sigma-Aldrich Anti-FLAG™ M2 Magnetic Beads **(A, B)**, Genscript Anti-DYKDDDDK G1 Affinity Resin **(C, D)**, Sigma-Aldrich Anti-FLAG M2 Affinity Gel **(C, D)** and MBL Anti-DDDDK-tag mAb-Magnetic Agarose **(A, B)** are shown. The comparison of the starting lysate and elution fractions show effective capture and elution of DYKDDDDK-tagged protein with minimal background by the Pierce supports compared to other suppliers.

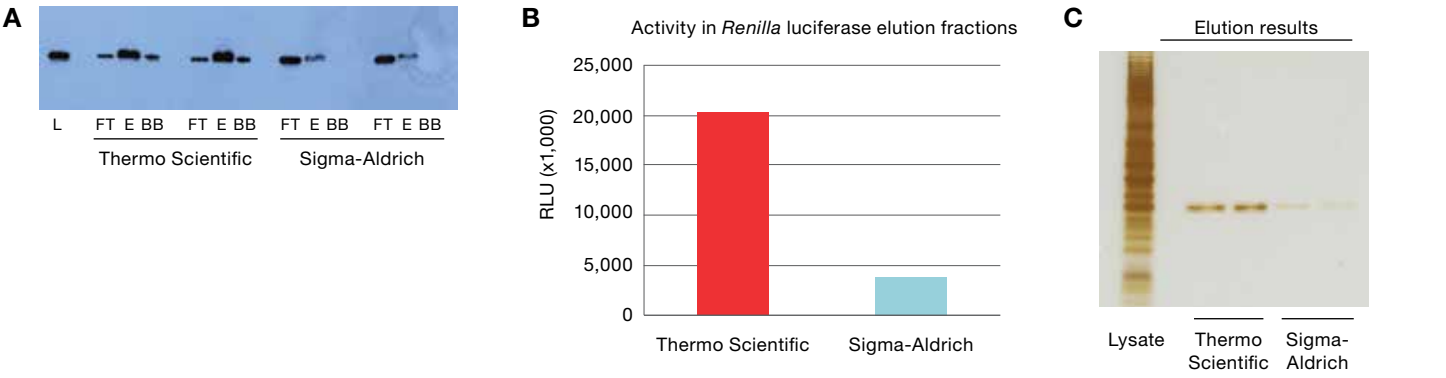


Figure 2. Comparison of protein purification results between Pierce Anti-DYKDDDDK Magnetic Agarose and another supplier. C-terminal DYKDDDDK-tagged green *Renilla* luciferase protein was expressed using the Thermo Scientific™ 1-Step Human High-Yield Maxi IVT Kit and immunoprecipitated using Pierce Anti-DYKDDDDK Magnetic Agarose or Sigma-Aldrich Anti-FLAG M2 Magnetic Beads using the Thermo Scientific™ KingFisher™ Flex Purification System. Tagged proteins were competitively eluted with Pierce 3x DYKDDDDK Peptide and analyzed by western blot **(A)**, Thermo Scientific™ Pierce™ *Renilla* Luciferase Glow Assay **(B)**, and silver staining **(C)**. Comparison of the starting lysate (L), flow-through (FT), elutions (E), and bead-boiled samples (BB) show effective capture and elution of DYKDDDDK-tagged proteins with no background. Correlation of protein and activity levels indicate that a high level of green *Renilla* luciferase activity is maintained after purification and competitive peptide elution.

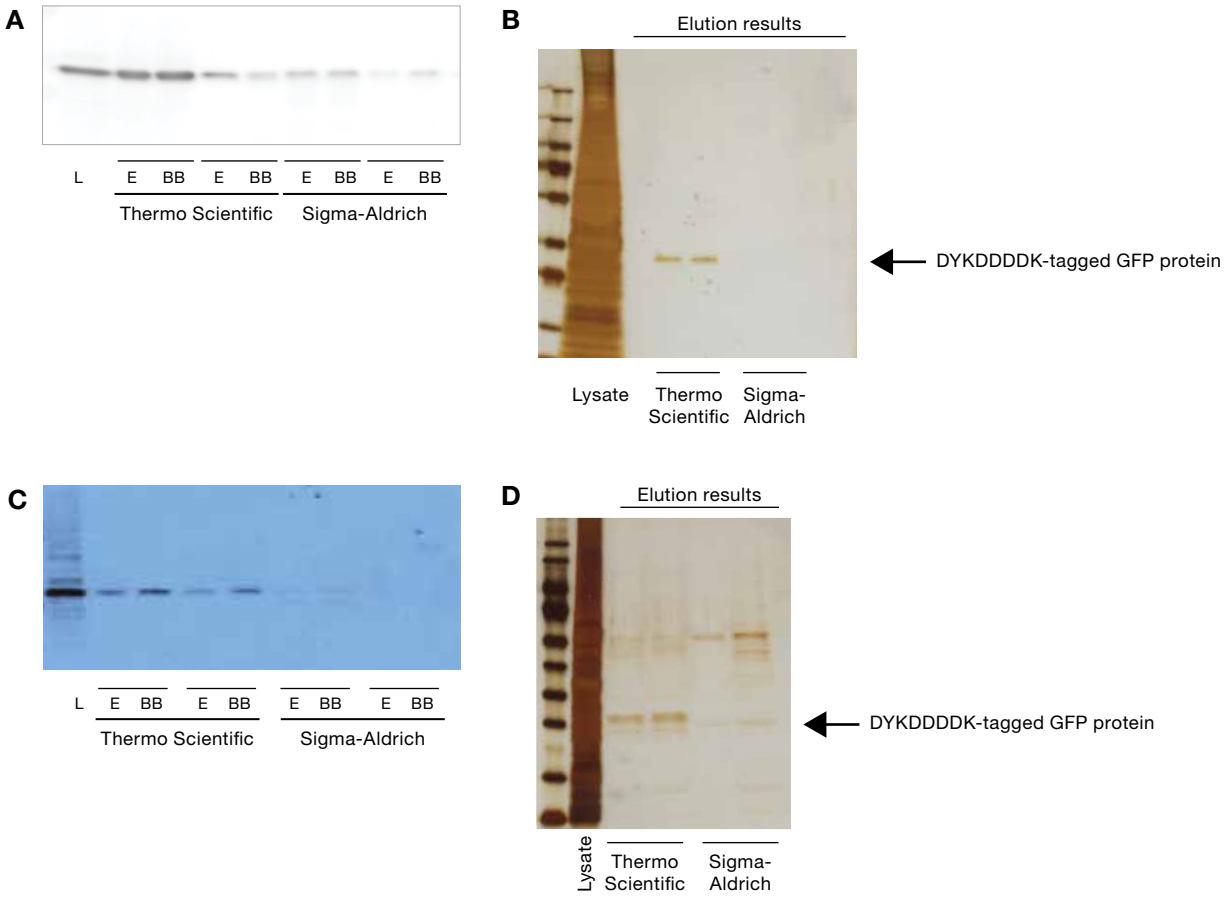


Figure 3. Comparison of capture efficiency of low-abundance DYKDDDDK-tagged protein. N-terminal DYKDDDDK-tagged GFP protein (25 ng) was spiked into A549 lysate and captured using Pierce Anti-DYKDDDDK Magnetic Agarose and Sigma-Aldrich Anti-FLAG M2 Magnetic Beads using the KingFisher Flex Purification System **(A, B)**. Tagged proteins were eluted using Thermo Scientific™ Pierce™ IgG Elution Buffer. The same conditions were used to compare the Pierce Anti-DYKDDDDK Affinity Resin with Sigma-Aldrich™ Anti-FLAG M2 Affinity Gel **(C, D)**, and results were analyzed by western blot **(A, C)** and silver staining **(B, D)**. Comparison of the starting lysate (L), elutions (E), and bead-boiled samples (BB) show effective capture and elution of DYKDDDDK-tagged proteins with minimal background by the Pierce supports compared to the other supplier.

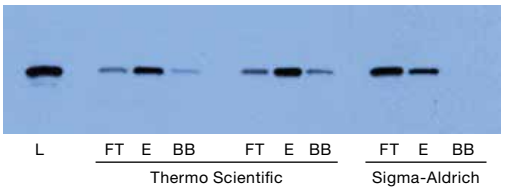


Figure 4. Comparison of protein purification results using Pierce Anti-DYKDDDDK Affinity Resin and another supplier. C-terminal DYKDDDDK-tagged GFP protein was expressed using the 1-Step Human High-Yield Maxi IVT Kit and immunoprecipitated using Pierce Anti-DYKDDDDK Affinity Resin or Sigma Anti-FLAG M2 Affinity Gel. Tagged proteins were competitively eluted with Pierce 3x DYKDDDDK Peptide and analyzed by western blot. Comparison of the starting lysate (L), elutions (E), and bead-boiled samples (BB) show effective capture and elution of DYKDDDDK-tagged proteins.

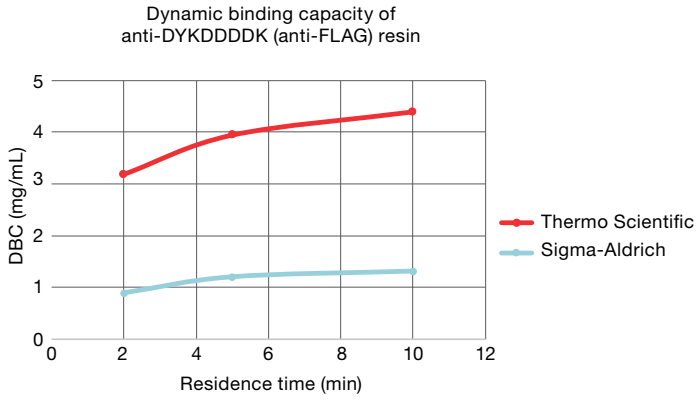


Figure 5. Dynamic binding capacity (DBC) versus residence time. Pierce Anti-DYKDDDDK Affinity Resin and Sigma Anti-FLAG M2 Affinity Gel were packed into 1 mL columns (0.5 cmD x 5 cmL) and loaded with purified DYKDDDDK-TurboGFP-His (1 mg/mL) in 100 mM phosphate, 150 mM NaCl, pH 7.2 (PBS) under a variety of residence times (150 cm/hr, 60 cm/hr, and 30 cm/hr) until a 10% breakthrough was achieved as measured by A_{280}.

Pierce Anti–c-Myc Magnetic Beads and Agarose Resin

Highly specific immobilized antibody for c-Myc epitope–tagged protein purification

Thermo Scientific™ Pierce™ Anti–c-Myc Magnetic Beads and Agarose resin are ideal for the high-affinity purification of recombinant c-Myc–tagged proteins expressed in cell-free, bacterial, yeast, insect, and mammalian expression systems. The Myc tag, derived from the c-Myc protein, is a popular epitope tag for detecting the expression of recombinant proteins and may be fused to either the N-terminus or C-terminus of a protein.

The anti–c-Myc antibody that is covalently immobilized to these base supports is a highly specific mouse IgG1 monoclonal antibody (clone 9E10) that recognizes the c-Myc epitope tag (EQKLISEEDL) derived from the human *MYC* oncogene (p62 *MYC*). The agarose support is a Thermo Scientific™ Pierce™ Superflow™ 6 resin, a highly crosslinked 6% agarose resin that can be packed into gravity purification columns, spin purification columns, or cartridges for FPLC instruments. The blocked magnetic beads (1 µM) can be used manually with a magnetic stand, as well as with automated platforms, such as KingFisher instruments, for high-throughput workflows.

The c-Myc–tagged protein is easily eluted from the resin after a few simple washing steps, using 0.1 M glycine (pH 2.0–2.8), 3 M NaSCN, or 50 mM NaOH, depending on the downstream application of the purified protein. For the elution of highly functional proteins, Thermo Scientific™ Pierce™ c-Myc Peptide can also be used to elute the c-Myc–tagged protein. Anti–c-Myc antibody may be used to detect the presence of the tagged protein by western blot.

In experiments with a c-Myc–tagged fusion protein (26 to 29 kDa), the resin provided a binding capacity up to 144 nmol protein per mL of settled resin. The anti–c-Myc magnetic beads have a binding capacity greater than 100 µg of c-Myc–tagged GST (26 kDa) per mL of bead suspension. Both supports are available in convenient kits for immunoprecipation (IP) and co-IP applications (Figure 6).

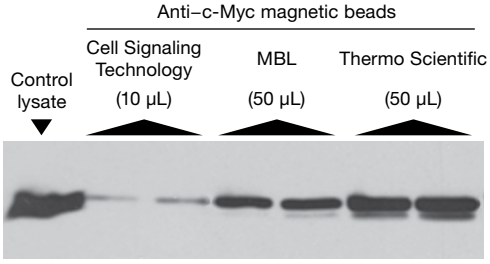


Figure 6. Better immunoprecipitation results with Pierce Anti–c-Myc Magnetic Beads. Green *Renilla* luciferase c-Myc fusion protein was expressed in 293T cells. For IP, identical aliquots of the cell lysate were incubated in duplicate for one hour at room temperature with anti–c-Myc magnetic beads from each manufacturer. For all conditions, IP products were eluted identically using low-pH buffer. Eluted fractions (25 µL each) were separated by 12% SDS-PAGE, transferred to PVDF membranes, and detected via anti–c-Myc antibody, goat anti-mouse secondary antibody, and chemiluminescent substrate.

Pierce Anti-HA Magnetic Beads and Agarose Resin

Highly specific immobilized antibody for HA-tagged protein purification

Thermo Scientific™ Pierce™ Anti-HA Magnetic Beads and Agarose resin are ideal for the immunopurification and immunoprecipitation of HA-tagged proteins expressed in cell-free, bacterial, yeast, and mammalian expression systems. The HA (hemagglutinin) tag is derived from the human influenza virus HA protein. It has been extensively used as a general antibody epitope tag and is well characterized. HA-tag antibodies provide a dependable method for the detection and purification of tagged target proteins without a protein-specific antibody or probe.

Pierce Anti-HA Agarose and Pierce Anti-HA Magnetic Beads consist of highly specific monoclonal anti-HA antibodies that recognize the HA-eptiope tag (YPYDVPDYA) derived from the human influenza HA protein. The agarose support is a crosslinked 4% beaded agarose. The blocked magnetic beads can be used manually with a magnetic stand, as well as with automated platforms, such as KingFisher instruments, for high-throughput workflows. Both supports are available in convenient kits for immunoprecipation (IP) and co-IP applications (Figure 7).

HA-tagged proteins are easily eluted from the supports using 0.1 M glycine (pH 2.0–2.8), 3 M NaSCN, or 50 mM NaOH, depending on the downstream application of the purified protein. For gentle elution, Thermo Scientific™ HA Synthetic Peptide is available for use in neutralization and to competitively elute HA-tagged fusion proteins from immobilized anti-HA magnetic beads and resin.

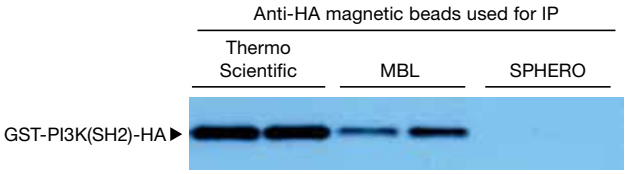


Figure 7. Better immunoprecipitation results with Thermo Scientific™ Pierce™ Anti-HA Magnetic Beads. Using a KingFisher Flex Purification System with 96 deep-well plates, 25 µL of Pierce Anti-HA Magnetic Beads, Anti-HA-tag Magnetic Beads (MBL International Corp.), and SPHERO™ Rabbit Anti-HA Magnetic Beads (Spherotech Inc.) were used to immunoprecipitate GST-PI3K(SH2)-HA from 50 µg of *E. coli* lysate in duplicate. Captured protein was eluted with 0.1 M glycine, pH 2.0, and then resolved by SDS-PAGE and analyzed by western blot for the HA-tagged protein.

His- and GST-tagged protein purification using affinity ligands

The polyhistidine tag is a sequence of 5–9 histidine amino acids attached to the terminus of a target protein. The polyhistidine tag is purified using immobilized metal affinity chromatography (IMAC). For histidine tag purification, either nickel or cobalt is immobilized onto a solid support. While the two metals can be used interchangeably, typically nickel has a higher binding capacity, whereas cobalt binds less nonspecific protein to deliver a purer final protein. Although the small polyhistidine tag is less likely to interfere with the target protein's structure and function, it can only be removed by the insertion of site-specific protease sequences. In addition, the polyhistidine tag is not recommended for proteins that contain metal ions.

Glutathione-*S*-transferase (GST) is a 26 kDa endogenous enzyme found in both prokaryotes and eukaryotes. GST possess numerous tyrosine residues in its binding pocket, and one of these tyrosines forms hydrogen bonds with the substrate glutathione to create a stable complex. The amino acid sequence for the enzyme GST can be cloned into either the C- or N-terminus of the target gene.

GST makes an ideal affinity tag because of its strong affinity to reduced glutathione and its potential to increase the solubility of target protein. This strong interaction has been used to selectively extract recombinant proteins. In this strategy, glutathione is immobilized onto a solid support. After binding of the GST-tagged fusion protein to the immobilized glutathione agarose, excess reduced glutathione is introduced (typically between 10–50 mM) to competitively elute off the target protein. Unlike IMAC, purification cannot be performed under denaturing conditions, but can promote the proper folding of recombinant proteins, although this larger tag may interfere with the structure and function of the target protein by forming dimers via the GST tag.

We offer several ligands for the purification of His-tagged proteins: nickel (as Ni-NTA or Ni-IDA) and cobalt. For the purification of GST-fusion proteins, we provide immobilized glutathione. Our affinity ligands for His- and GST-tagged proteins are provided on magnetic beads and other resins, and are available in multiple formats to accommodate a variety of needs, from high-throughput screening to batch- and pilot-scale purification (Tables 4 and 5).

Highlights:

- **Specific**—highly specific affinity ligands enable high yield and high purity for high-throughput to pilot-scale protein purification
- **Low nonspecific binding**—stable beads and target-specific affinity ligands minimize nonspecific binding
- **Scalable**—available in larger package sizes for purification scale-up and more economical pricing
- **Versatile**—multiple resin formats are available that can be used in spin or gravity columns as well as in fast protein liquid chromatography (FPLC) cartridges
- **Automation-friendly**—magnetic agarose or beads recommended for high-throughput applications
- **Convenient**—reagents to elute and detect tagged fusion proteins are available separately

Table 4. Overview of His-tagged protein purification beads and resins.

	Pierce Ni-NTA Magnetic Agarose Beads	Probond Nickel Chelating Resin	HisPur Ni-NTA Agarose Resin	HisPur Ni-NTA Superflow Resin	HisPur Cobalt Agarose Resin	HisPur Cobalt Superflow Resin
Bead or resin size	10–40 µm	45–165 µm	45–165 µm	60–160 µm	45–165 µm	60–160 µm
Static binding capacity*	>70 mg/mL	1–5 mg/mL	~60 mg/mL	>60 mg/mL	≥15 mg/mL	>30 mg/mL
Dynamic binding capacity*	NA	ND†	18 mg/mL	20 mg/mL	ND†	>20 mg/mL
Maximum flow rate	NA	700 cm/hr	700 cm/hr	1,200 cm/hr	700 cm/hr	1,200 cm/hr
Support	Magnetite-embedded 6% agarose	6% agarose, highly cross-linked	6% agarose	6% agarose, highly crosslinked	6% agarose	6% agarose, highly crosslinked
Number of reuses	Not recommended	6	5	25	5	25
Formats available	Loose bead	Loose resin, kit	Loose beads, prepacked spin columns and kits, chromatography cartridges	Loose resin	Loose beads, prepacked spin columns and kits, chromatography cartridges	Loose resin
Recommended application scale	High-throughput batch	Batch	Batch	Batch, pilot	Batch	Batch, pilot

* See product pages for details.
† Not determined.

Table 5. Overview of GST-tagged protein purification beads and resins.

	Pierce Glutathione Magnetic Agarose Beads	Glutathione Agarose Resin	Glutathione Superflow Resin
Bead or resin size	10–40 µm	45–165 µm	60–160 µm
Static binding capacity*	>12 mg/mL	~40 mg/mL	~30 mg/mL
Dynamic binding capacity*	NA	~10.5 mg/mL	~10 mg/mL
Maximum flow rate	NA	800 cm/hr	1,200 cm/hr
Support	Magnetite-embedded 6% agarose	6% agarose	6% agarose, highly crosslinked
Number of reuses	Not recommended	5	25
Formats available	Loose bead	Loose beads, prepacked spin columns and kits, chromatography cartridges	Loose resin
Recommended application scale	High-throughput batch	Batch	Batch, pilot

* See product pages for details.

HisPur Ni-NTA and Cobalt Magnetic Beads and Resins

High-performance immobilized Ni-NTA and cobalt supports for his-tagged protein purification

Thermo Scientific™ HisPur™ Ni-NTA and Cobalt IMAC resins work by charge interactions with the nitrogen atoms on the 6x histidine amino acid side chain to bind and immobilize the histidine-tagged protein from a cell lysate. The incorporation of multiple histidine residues as an affinity tag is designed to improve this charge association. Because IMAC affinity for histidine residues is not dependent on the secondary structure of the protein, IMAC purification can be performed under denaturing conditions.

Once immobilized, imidazole is used to disrupt the charge attractions between the immobilized metal affinity chromatography resin and the histidine-tagged protein. The eluted histidine-tagged protein can be easily cleaned up using a desalting column or dialysis cassette to remove imidazole. Often trace amounts of imidazole are included in the protein binding and wash steps to compete with binding of endogenous proteins with multiple histidines.

We offer three different magnetic supports: Thermo Scientific™ HisPur™ Ni-NTA Magnetic Beads, Thermo Scientific™ Pierce™ Ni-NTA Magnetic Agarose Beads, and Invitrogen™ Dynabeads™ His-Tag Isolation Magnetic Beads. The magnetic agarose bead has a significantly higher binding capacity and is ideal for enriching or purifying low-abundance proteins. The Dynabeads His-Tag Isolation Magnetic Beads provides a magnetic bead option using a cobalt ligand.

Magnetic beads are optimized for protein enrichment from small-volume samples with low protein concentrations. These magnetic formats are optimized for automated assays using instrumentation such as the Thermo Scientific™ KingFisher™ Magnetic Particle Processor. This format is ideal for small- to batch-scale screening applications for microgram to milligram protein yields.

We offer three ligands on agarose supports: Thermo Scientific™ HisPur™ Ni-NTA Agarose Resin, Thermo Scientific™ HisPur™ Cobalt Agarose Resin, and Invitrogen™ ProBond™ Nickel-Chelating Resin and kits, which use an alternative Ni-chelating ligand, iminodiacetic acid (IDA). Optimized for laboratory-scale fusion protein purification, this base support (6% agarose) can be used for microscale preparations to columns ≤25 mL in volume. Agarose is less rigid than Superflow resin, and is therefore used at lower flow rates. Common tabletop microcentrifuges are often used for separation of the solid phase. This format is ideal for batch-scale applications resulting in milligram to gram protein yields.

We offer two ligands on Superflow supports: Thermo Scientific™ HisPur™ Ni-NTA Superflow Resin and Thermo Scientific™ HisPur™ Cobalt Superflow Resin. The Superflow resin is used for pilot- to process-scale purification at high flow rates. The highly crosslinked form of the resin imparts improved rigidity, enabling it to withstand higher pressure and flow rates without compressing. This makes it easy to scale up from laboratory- to pilot-scale purifications resulting in gram to low-kilogram protein yields.

Learn more at **thermofisher.com/histag-purification**

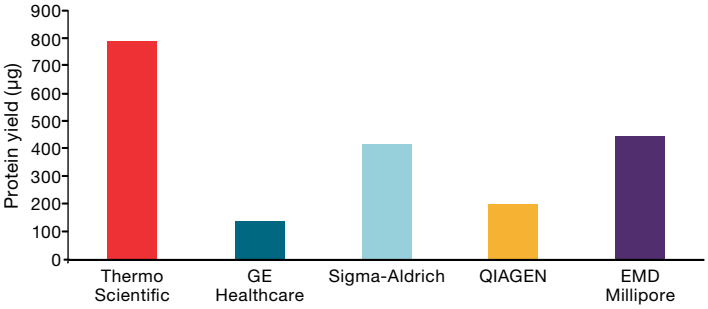


Figure 8. Comparison of protein yields between Pierce Ni-NTA Magnetic Agarose and products from other suppliers. Samples (0.5 mL) of 6xHis-tagged BirA protein were diluted with 0.5 mL binding buffer and purified manually with 25 mL settled beads. Respective suppliers' protocols were followed for their buffer compositions and volumes. Pierce Ni-NTA Magnetic Agarose had the highest yield compared to beads from the other suppliers.

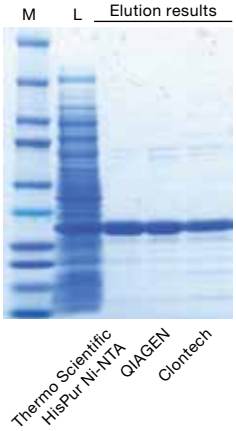
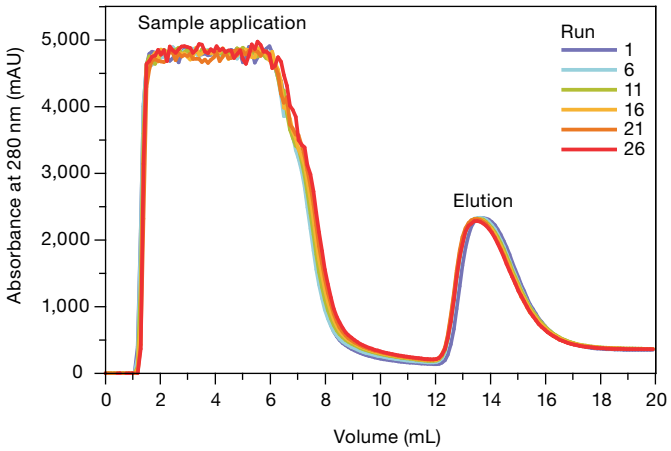


Figure 9. HisPur Ni-NTA Agarose resin performs as well as or better than other suppliers' nickel resins. Bacterial lysate (12 mg total protein) containing overexpressed 6xHis-GFP (green fluorescent protein) was applied to HisPur Ni-NTA Resin (Cat. No. 88221) (0.2 mL) and purified by the batch-bind method. The same amount of total protein was applied to QIAGEN and Clontech resins per the manufacturers' instructions. Gel lanes were normalized to equivalent volume. M = molecular weight marker; L = lysate load.



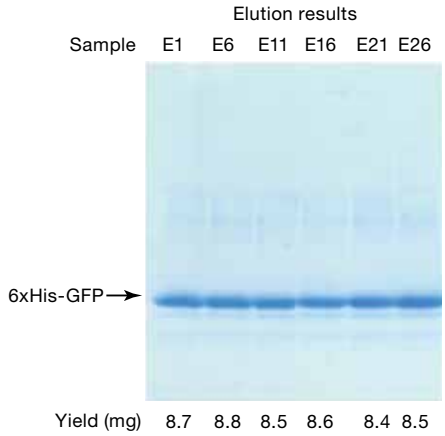


Figure 10. High reusability of HisPur Cobalt Superflow Agarose. 6xHis-GFP lysate (5 mL) was loaded onto an equilibrated 1 mL column (column diameter = 0.7 cm) packed with HisPur Cobalt Superflow Agarose at a flow rate of 1 mL/min, washed with 5 column volumes (CV) of wash buffer containing 15 mM imidazole, and eluted with 10 CV of elution buffer containing 150 mM imidazole. Protein yields were measured in the collected samples by absorbance at 280 nm throughout the FPLC run (chromatogram). The column was regenerated with 10 CV of MES-buffered saline (20 mM MES pH 5.0, 300 mM NaCl) to remove imidazole, and then 10 CV of ultrapure water, followed by equilibration with 10 volumes of binding buffer containing 5 mM imidazole. A lysate challenge was included every fifth cycle for a total of 20 blank runs and six lysate challenges (runs 1, 6, 11, 16, 21, and 26). 6xHis-GFP yield and purity were similar for all lysate challenges as seen by protein estimation with the Thermo Scientific™ Pierce™ 660 nm Protein Assay Kit and SDS-PAGE.

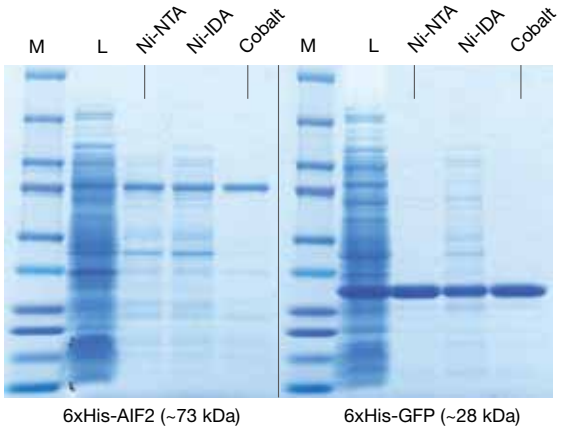


Figure 11. High-performance purification of different-sized proteins using HisPur Ni-NTA and HisPur Cobalt Agarose Resins. Bacterial lysate containing overexpressed 6xHis-AIF2 (6 mg total protein) or 6xHis-GFP (4 mg total protein) was applied to HisPur Ni-NTA Agarose Resin (0.2 mL) and purified by the batch-bind method. The same amount of total protein was applied to Ni-IDA and HisPur Cobalt Agarose Resins and purified according to the manufacturer's instructions. Gel lanes were normalized to equivalent volume. M = molecular weight markers; L = lysate load; and FT = flow-through. HisPur Ni-NTA and HisPur Cobalt Agarose Resins maximize yield and purity, respectively.

Pierce Glutathione Magnetic Agarose and Resins

High-performance immobilized glutathione supports for GST-tagged protein purification

In contrast to polyhistidine purification, GST purification requires the target protein to maintain native tertiary structure. Additionally, the GST tag is quite large (26 kDa) compared to the six histidines that comprise a typical polyhistidine tag. To circumvent the problems associated with a large fusion protein tag, selective proteases are used to cleave the GST tag from the GST-fusion protein if needed. These proteases include factor Xa or thrombin.

Reduced glutathione (GSH), when immobilized as a ligand to agarose or other chromatography supports, enables high-yield and high-quality purification of recombinant proteins expressed as fusions with glutathione *S*-transferase (GST). GST-fusion proteins are purified with high yield because of the 12-atom GSH linker, which minimizes steric hindrance.

We offer Thermo Scientific™ Pierce™ Glutathione Magnetic Agarose Beads for protein enrichment from small-volume samples (Figure 12). This format is ideal for small- to batch-scale screening applications for microgram–milligram yields and can be automated using instrumentation such as the KingFisher Magnetic Particle Processor.

Thermo Scientific™ Pierce™ Glutathione Agarose is optimized for laboratory-scale fusion protein purification. This base support (6% agarose) can be used for microscale preparations with columns ≤25 mL in volume. Agarose is less rigid than Superflow resin, and is therefore used at lower flow rates. Common tabletop microcentrifuges are often used for separation of the solid phase. This format is ideal for batch-scale applications resulting in milligram to gram protein yields.

Thermo Scientific™ Pierce™ Glutathione Superflow resin is recommended for pilot- to process-scale purification at high flow rates. The highly crosslinked form of the resin imparts improved rigidity, enabling it to withstand higher pressure and flow rates without compressing. This makes it easy to scale up from laboratory- to pilot-scale purifications resulting in gram to low-kilogram protein yields.

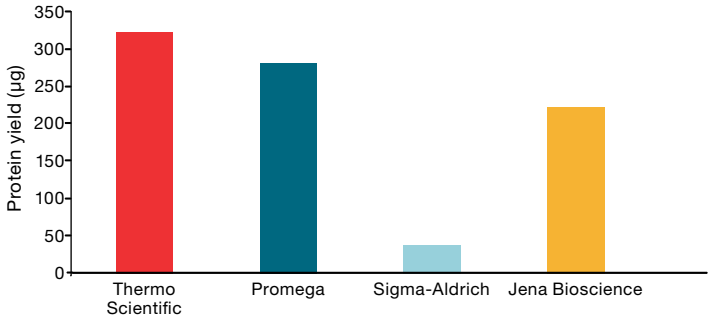


Figure 12. Comparison of protein yields between Pierce Glutathione Magnetic Agarose Beads and products from other suppliers. Samples (0.25 mL) of GST-RalGDS were diluted with 0.25 mL binding buffer and purified manually with 25 µL settled beads. Respective suppliers' protocols were followed for their buffer compositions and volumes. Pierce Glutathione Magnetic Agarose Beads had the highest yield compared to beads from the other suppliers.

Learn more at **thermofisher.com/gst-purification**

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Ordering information

Product	Quantity	Cat. No.
Epitope-tagged protein purification using immunoaffinity ligands		
Pierce Anti-DYKDDDDK Magnetic Agarose	1 mL	A36797
	5 mL	A36798
	50 mL	A36799B
Pierce Anti-DYKDDDDK Affinity Resin	1 mL settled	A36801
	5 mL settled	A36803
	50 mL settled	A36804
Pierce 3x DYKDDDDK Peptide	5 mg	A36805
	5 x 5 mg	A36806
EKMax Enterokinase	250 units	E18002
Pierce Anti-c-Myc Agarose	2 mL	20168
Pierce c-Myc-Tag IP/Co-IP Kit	25 reactions	23620
Pierce Anti-c-Myc Magnetic Beads	1 mL	88842
Pierce c-Myc-Tag Magnetic IP/Co-IP Kit	40 reactions	88844
Pierce c-Myc Peptide	5 mg	20170
Pierce Anti-HA Agarose	1 mL	26181
Pierce HA-Tag IP/Co-IP Kit	25 reactions	26180
Pierce Anti-HA Magnetic Beads	1 mL	88826
Pierce HA-Tag Magnetic IP/Co-IP Kit	40 reactions	88838
HA Synthetic Peptide	5 mg	26184

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His-tagged protein purification using IMAC		
HisPur Ni-NTA Magnetic Beads	2 mL	88831
Pierce Ni-NTA Magnetic Agarose Beads	1 mL	78605
Dynabeads His-Tag Isolation and Pulldown	2 mL	10103D
HisPur Ni-NTA Agarose Resin	10 mL	88221
	100 mL	88222
	500 mL	88223
HisPur Ni-NTA Agarose Chromatography Cartridges, 1 mL	5 cartridges	90098
HisPur Ni-NTA Agarose Chromatography Cartridges, 5 mL	2 cartridges	90099

Product	Quantity	Cat. No.
His-tagged protein purification using IMAC (cont.)		
HisPur Ni-NTA Superflow Agarose	10 mL	25214
	50 mL	25215
	250 mL	25216
	1 L	25217
ProBond Nickel-Chelating Resin	50 mL	R80101
	150 mL	R80115
HisPur Cobalt Agarose Resin	10 mL	89964
	100 mL	89965
	500 mL	89966
HisPur Cobalt Agarose Chromatography Cartridges, 1 mL	5 cartridges	90093
HisPur Cobalt Agarose Chromatography Cartridges, 5 mL	2 cartridges	90094
HisPur Cobalt Superflow Agarose	10 mL	25228
	50 mL	25229
	100 mL	25230
	500 mL	25231

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GST-tagged protein purification using immobilized glutathione		
Pierce Glutathione Magnetic Agarose Beads	1 mL	78601
Pierce Glutathione Agarose	10 mL	16100
	100 mL	16101
	500 mL	16102
Pierce Glutathione Chromatography Cartridges, 1 mL	5 cartridges	16109
Pierce Glutathione Chromatography Cartridges, 5 mL	2 cartridges	16110
Pierce Glutathione Superflow Agarose	10 mL	25236

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