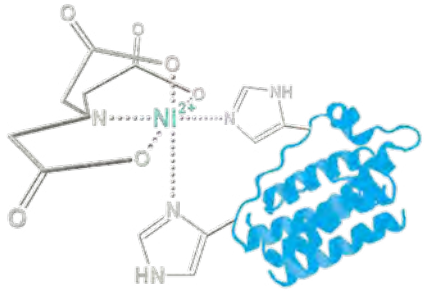

# fusion protein purification

## screening • batch • pilot • process

Thermo SCIENTIFIC

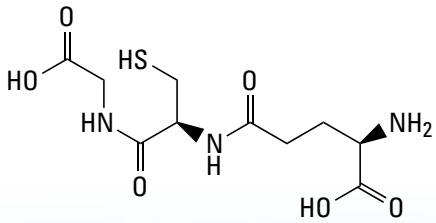
# exceptional purification

**The expression and purification of recombinant proteins** is essential to protein regulation, structure and function studies. The majority of recombinant proteins are expressed as fusion proteins with short affinity tags, such as polyhistidine (6xHis) or glutathione S-transferase (GST). These tags allow researchers to selectively extract a protein of interest from the thousands of other proteins found in the cell. Recombinant His-tagged proteins are purified using immobilized metal affinity chromatography (IMAC), consisting of chelating resins charged with either nickel or cobalt ions that coordinate with the histidine side chains. Reduced glutathione resins are used to purify GST-tagged proteins.




### Polyhistidine (His) Tag

The polyhistidine tag is a sequence of five to nine histidine amino acids cloned into either the C- or N-terminus of the target gene. His-tagged proteins are purified using immobilized metal affinity chromatography (IMAC). Nickel or cobalt is immobilized onto a solid chromatography resin to selectively bind His-tagged proteins from a cell lysate. In general, nickel is used for higher protein yield while cobalt is used for higher purity preparations.




**Glutathione (GSH)**
**MW 307.32**

### Glutathione S-Transferase Tag

The high affinity between GST and reduced glutathione (GSH) is used to selectively extract recombinant proteins. The amino acid sequence for the enzyme GST is cloned into either the C- or N-terminus of the target gene. Reduced glutathione is immobilized onto a solid chromatography resin to selectively bind GST-tagged proteins from a cell lysate.

**Thermo Scientific™ Affinity Purification Resins.**

| Affinity Tag | | 6xHis-Tag | | GST-Tag |
|---|---|---|---|---|
| Ligand | | $Ni^{2+}$ **Nickel** | $Co^{2+}$ **Cobalt** | GSH **Reduced Glutathione** |
| Resin Type | **Magnetic** | HisPur Ni-NTA Magnetic Beads | | Magnetic Glutathione Beads |
| | **Agarose** | HisPur Ni-NTA Agarose Resin | HisPur Cobalt Agarose Resin | Pierce Glutathione Agarose |
| | **Superflow** | HisPur Ni-NTA Superflow Agarose | HisPur Cobalt Superflow Agarose | Glutathione Superflow Agarose |

Purification of fusion proteins at both small and large scales requires robust methods that result in high yields and purity. We offer a broad range of products for affinity purification of recombinant proteins containing polyhistidine or GST tags. Resins are available for purification of His-tagged proteins using cobalt or nickel IMAC. For GST-tagged protein purification, we offer immobilized glutathione resins.

These affinity resins are available on the following solid supports:

- Magnetic beads for protein enrichment from small-volume samples with low protein concentrations
- 6% agarose resin for laboratory-scale applications
- Superflow agarose for large-scale purifications

In addition, our resins are supplied in a variety of sizes and packaging formats to suit many protein purification scales and applications.

# choosing the right resin

**Resin selection** is largely based on the scale of protein purification you are performing. Magnetic beads or agarose resin can be used for screening experiments involving a large number of proteins. Agarose is generally used for smaller scale purification at moderate flow rates, while superflow resin is optimized for larger scale purifications at high flow rates.

## When to use Magnetic Beads

Magnetic beads are optimized for protein enrichment from small-volume samples with low protein concentrations. Sold only in slurry format, these beads are optimized for automated assays using instrumentation such as the Thermo Scientific™ KingFisher™ Magnetic Particle Processor. Magnetic beads are not ideal for purification, rather they are better suited for screening applications.

**Magnetic affinity purification beads are ideal for:**

- Mutational analysis
- High-throughput screening
- Pull-down assays

**Magnetic Bead Characteristics**

| | |
|---|---|
| **Bead Size** | ~1µm |
| **Bead Composition** | Magnetite-coated polymeric beads blocked and covalently coated with various ligands |
| **Flow Rate** | Not applicable |
| **Storage Conditions** | Water |

**Select your resin based on purification scale and application.**

| Scale | Screening | Batch | Pilot | Process |
|---|---|---|---|---|
| **Technique** | • automated particle processor<br>• 96-well spin plates | • gravity flow<br>• spin columns | FPLC at medium flow rates | FPLC at high flow rates |
| **Yield** | microgram | milligram | milligram to gram | gram to kilogram |
| **Application** | • high-throughput screening<br>• interaction studies<br>• mutational analysis | • functional assays<br>• structural analysis | structural analysis | bulk production |
| **Recommended resin type** | **Magnetic** | | | |
| | | **Agarose** | | |
| | | | **Superflow** | |

## When to use 6% Agarose Resin

Optimized for laboratory-scale fusion protein purification, 6% agarose can be used for microscale preparations to columns ≤ 25mL in volume. Agarose is less rigid than superflow resin and, therefore, is used at lower flow rates. Common table top microcentrifuges are often used for separation of the solid phase.

**Agarose affinity purification resins are ideal for:**

- Small sample volumes
- Low to moderate flow rates
- Batch, gravity or spin purification formats
- Co-purifying multiple proteins in parallel

6% Agarose Resin Characteristics

| | |
|---|---|
| **Bead Size** | 45-150µm |
| **Flow Rate** | 800cm/hr |
| **Agarose Content** | 6% |
| **Crosslinking** | Moderate |
| **Maximum Pressure** | 0.35MPa |
| **Recommended Centrifuge Speed** | 700 x *g* |
| **Molecular Weight Exclusion Limit** | > 4 x $10^6$ Daltons |
| **Storage Conditions** | 20% ethanol, 4°C |

## When to use Superflow Resin

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 high pressure and flow rates without compressing. This makes it easy to scale up from laboratory to industrial scale purifications.

**Superflow affinity purification resins are ideal for:**

- Large sample volumes
- Moderate to high flow rates
- Packing custom columns
- Fast protein liquid chromatography (FPLC) instruments

Superflow Resin Characteristics

| | |
|---|---|
| **Bead Size** | 60-160µm |
| **Flow Rate** | 1,200cm/hr |
| **Agarose Content** | 6% |
| **Crosslinking** | High |
| **Maximum Pressure** | 0.65MPa |
| **Molecular Weight Exclusion Limit** | 6 x $10^6$ Daltons |
| **Storage Conditions** | 20% ethanol, 4°C |

# different formats
## different applications

**Thermo Scientific™ Protein Purification Resins** are available in different formats to suit a variety of applications. Select the format that best fits your experiment.

### Slurry

Loose resin in a storage buffer containing a preservative

Select this format for:

- Packing columns
- Larger-scale purification
- Custom assays

### Spin Columns

Microcentrifuge columns packed with 0.2mL, 1mL or 3mL of resin

Select this format for:

- Small volume preparations
- Multiple preparations
- Pull-down assays
- Fast purification using any common centrifuge


### Purification Kits

Complete kits with pre-packed spin columns and validated buffers for optimized protein purification

Select this format for:

- All reagents needed for purification
- The best yield
- An optimized protocol

## FPLC Cartridges

FPLC cartridges pre-packed with affinity media

Select this format for:

- Automated purification
- Low to moderate flow rate
- Use with common instrumentation such as the AKTA™ Instrument

## 96-well Spin Plates

96-well filter plates pre-packed with affinity resin

Select this format for:

- High-throughput purification
- Screening experiments


## Bulk Resin

Large volume and custom packaging of any of our affinity resins

Select this format for:

- Large-scale purification
- Discounted pricing for large-volume orders
- Custom packaging formats or QC testing

# Ni-NTA Magnetic Beads

**The Thermo Scientific™ HisPur™ Ni-NTA Magnetic Beads** are high-capacity nickel-IMAC beads for affinity purification of His-tagged fusion proteins in manual or automated formats. These beads are chemically blocked to reduce background and nonspecific binding. The blocked magnetic bead surface is derivatized with the nitrilotriacetic acid (NTA) chelation moiety and loaded with divalent nickel ions ($Ni^{2+}$). The immobilized metal affinity chromatography (IMAC) beads provide high binding capacity with very low background. The HisPur Ni-NTA Magnetic Beads can be used both manually with a magnetic stand as well as with automated platforms such as the Thermo Scientific™ KingFisher™ Instruments for high-throughput needs.

## Highlights

- **High capacity** – equivalent or higher binding capacity than Ni-NTA magnetic beads from other suppliers
- **Low nonspecific binding** – the bead surface is pre-blocked and the protocol provides optimized buffers for purification
- **Fast** – protocol is completed in less than one hour
- **Scalable** – process microliter to milliliter sample volumes
- **Versatile** – purify proteins using native or denaturing conditions
- **Reagent compatible** – can be used with common cell lysis reagents and a variety of buffer additives
- **Multiple formats** – protein coupling to the beads and downstream applications can be performed both manually and on an automated platform (e.g., KingFisher Instruments)

**Table 1. Characteristics of Thermo Scientific HisPur Ni-NTA Magnetic Beads.**

| | |
|---|---|
| **Composition** | $Ni^{2+}$ loaded on nitrilotriacetic acid that has been covalently coupled to the beads |
| **Mean Diameter** | 1µm (nominal) |
| **Density** | 2.0g/$cm^3$ |
| **Bead Concentration** | 12.5mg/mL in 20% ethanol |
| **Binding Capacity** | ≥ 40µg of 6xHis-tagged GFP/mg of beads;<br>≥ 500µg 6xHis-tagged GFP/mL of beads |

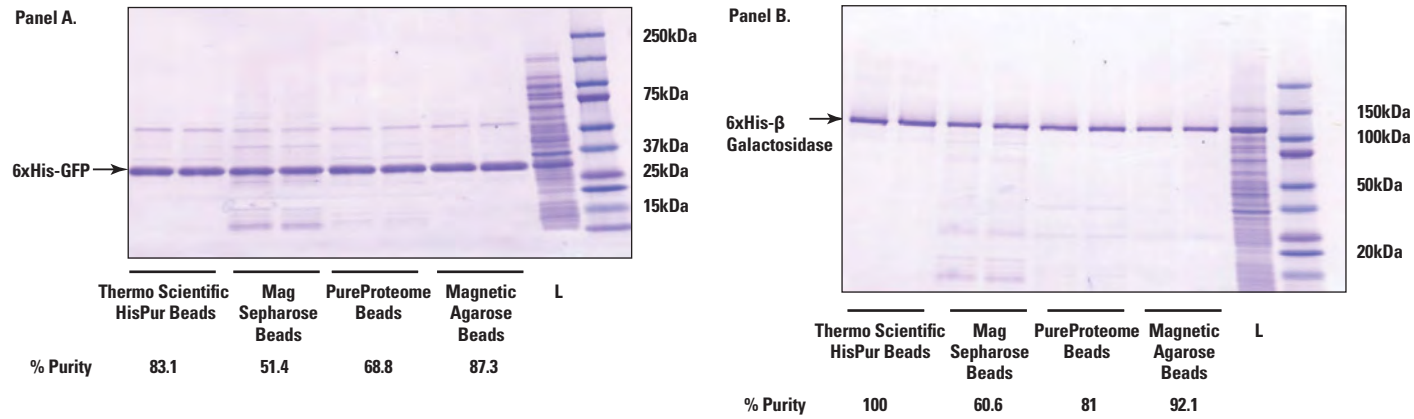



**Figure 1. Superior performance of Thermo Scientific HisPur Ni-NTA Magnetic Beads when compared to magnetic beads from other suppliers.** Bacterial lysate (100mg total protein) containing over-expressed 6xHis-GFP (**Panel A**) or over-expressed 6xHis-β Galactosidase (**Panel B**) was applied to 0.5mg of HisPur Ni-NTA Magnetic Beads, Mag Sepharose (GE Healthcare), PureProteome™ (Millipore) and Magnetic Agarose (Qiagen) Ni-NTA bead products. All samples were run in duplicate, and the beads were processed using the Thermo Scientific protocol with buffers recommended by the manufacturers. For the HisPur Ni-NTA Magnetic Beads, the amount of imidazole in the equilibration, wash and elution buffers was 30mM, 50mM and 150mM, respectively. All three buffers contained 100mM sodium phosphate and 600mM sodium chloride. Binding was performed with all samples for 30 minutes. The beads were collected on a magnetic stand and the flow-throughs were saved for analysis. The beads were then washed twice and bound protein was eluted for 2 x 15 minutes with elution buffer. The eluates were combined, resolved on an SDS-PAGE gel and stained with Thermo Scientific™ Imperial™ Stain (Product # 24615). For purification of 6xHis-GFP, comparable yields and comparable % purity were observed for HisPur Ni-NTA and Qiagen Ni-NTA magnetic beads. HisPur Ni-NTA Magnetic Beads showed higher yield and purity than Qiagen Ni-NTA beads in the purification of 6xHis-β Galactosidase. Millipore and GE Ni-NTA magnetic beads gave lower purity and lower yield than Thermo Scientific beads in both purifications. Purity analyses were performed on a Thermo Scientific™ MYECL™ Imager with Thermo Scientific™ MYIMAGEANALYSIS™ Software. Purity was determined by measuring the ratio of the background corrected 6xHis-tagged protein band of interest to the sum of all bands and multiplying by 100%. **L** = lysate.

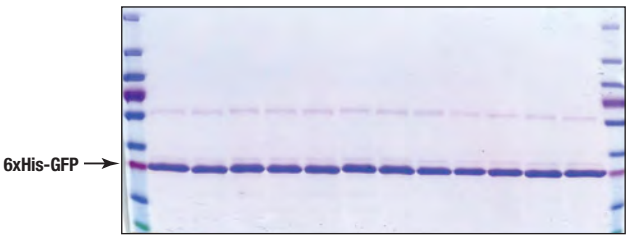



**Figure 2. Thermo Scientific HisPur Ni-NTA Magnetic Beads deliver consistent yield.** His-tag protein purification was performed in a 96-well plate using the Thermo Scientific™ KingFisher™ Flex Instrument. In each well, 100µg of *E. coli* lysate expressing 6xHis-GFP protein was added to 0.5mg of HisPur Ni-NTA Magnetic Beads. Eluted protein was analyzed by SDS-PAGE stained with Imperial Protein Stain (Product # 24615) to determine well-to-well consistency in protein recovery. The variance between samples is measured at less than 15%.


## Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 88831 | HisPur Ni-NTA Magnetic Beads | 2mL |
| 88832 | HisPur Ni-NTA Magnetic Beads | 10mL |

# $Ni^{2+}$ polyhistidine purification with nickel

## Ni-NTA Agarose Resin

**Thermo Scientific™ HisPur™ Ni-NTA Agarose Resin** is a high-capacity, high-performance nickel-IMAC resin for routine affinity purification of His-tagged fusion proteins. The specially prepared support consists of 6% beaded agarose derivatized with the nitrilotriacetic acid (NTA) chelation moiety and loaded with divalent nickel ions ($Ni^{2+}$). The immobilized metal affinity chromatography (IMAC) resin provides exceptional binding capacity and performance for recombinant His-tagged protein purification.

### Highlights

- **High capacity** – binds up to 60mg of 6xHis-tagged protein per milliliter of resin
- **Versatile** – purify proteins using native or denaturing conditions
- **Compatible** – use with Thermo Scientific™ Cell Lysis Reagents and a variety of buffer additives
- **Flexible** – available in multiple formats, including bulk resin, spin columns, chromatography cartridges and 96-well filter plates
- **Cost effective** – reuse the same batch of resin at least five times
- **Easy to use** – pre-formulated buffers available in kit formats

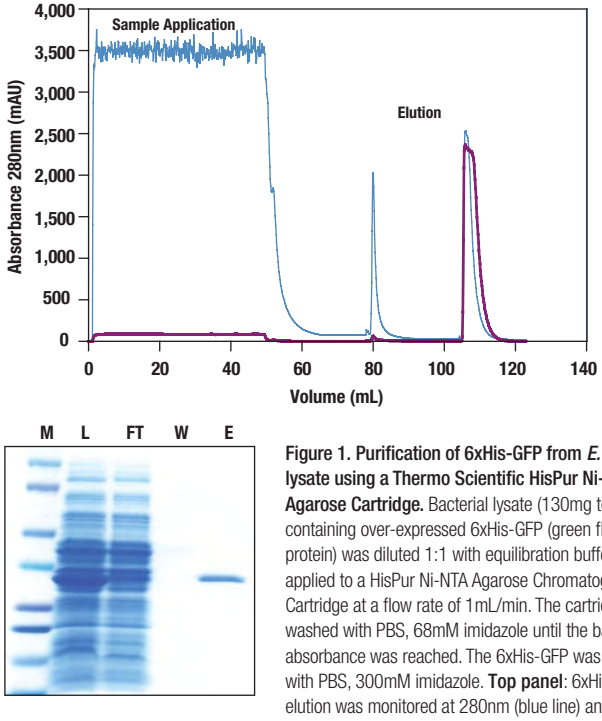



**Figure 1. Purification of 6xHis-GFP from *E. coli* lysate using a Thermo Scientific HisPur Ni-NTA Agarose Cartridge.** Bacterial lysate (130mg total protein) containing over-expressed 6xHis-GFP (green fluorescent protein) was diluted 1:1 with equilibration buffer and applied to a HisPur Ni-NTA Agarose Chromatography Cartridge at a flow rate of 1mL/min. The cartridge was washed with PBS, 68mM imidazole until the baseline absorbance was reached. The 6xHis-GFP was eluted with PBS, 300mM imidazole. **Top panel**: 6xHis-GFP elution was monitored at 280nm (blue line) and 485nm (purple line; GFP-specific). **Bottom panel**: Selected fractions were analyzed by SDS-PAGE. Gel lanes were normalized to equivalent volume. **M** = molecular weight markers; **L** = lysate load; **FT** = flow-through; **W** = wash; and **E** = elution.

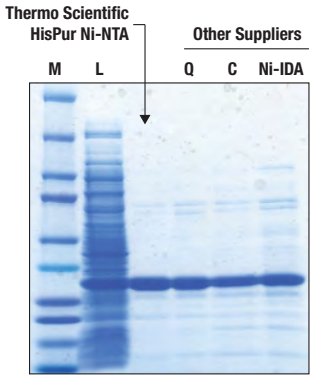
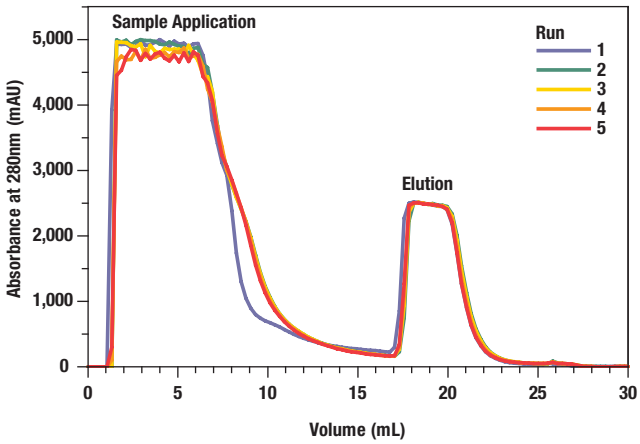



**Figure 2. Thermo Scientific HisPur Ni-NTA Agarose Resin performs as well as or better than other suppliers' nickel resins.** Bacterial lysate (12mg total protein) containing over-expressed 6xHis-GFP (green fluorescent protein) was applied to HisPur Ni-NTA Agarose Resin (0.2mL) and purified by the batch-bind method. The same amount of total protein was applied to Supplier Q (Qiagen), Supplier C (Clontech), and Ni-IDA resins per the manufacturers' instructions. Gel lanes were normalized to equivalent volume. **M** = molecular weight markers; **L** = lysate load.



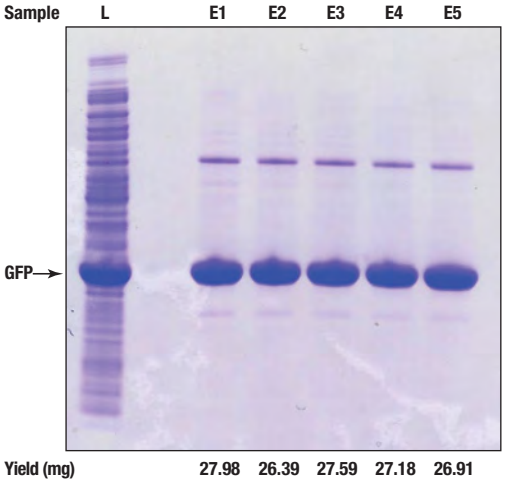




**Figure 3. Reuse Thermo Scientific HisPur Ni-NTA Agarose Resin at least five times without loss in protein purification efficiency.** Packed HisPur Ni-NTA Resin (1mL) was equilibrated with 5 column volumes (CV) of binding buffer (20mM phosphate, 300mM sodium chloride, 10mM imidazole, pH 7.4). GFP lysate (5mL) was injected at 1mL/min onto the column. The column was then washed with 10 CV of wash buffer (20mM phosphate, 300mM sodium chloride, 30mM imidazole, pH 7.4). The bound GFP was eluted using 10 CV of elution buffer (20mM phosphate, 300mM sodium chloride, 300mM imidazole, pH 7.4). Protein yields were measured in the collected samples by absorbance at 280nm throughout the FPLC run (chromatogram).The column was regenerated using 10 CV of 20mM MES, 300mM NaCl, pH 5. Protein yields were measured in the collected samples by absorbance at 280nm throughout the FPLC run (chromatogram). The column was regenerated and reused 5 times. The amount of protein in each elution was determined using the Thermo Scientific™ Pierce™ 660nm Protein Assay (Product # 22662) and 5µg of each eluate were loaded on the gel. The gel was stained with Imperial Stain for 1 hour and destained with water. Purity analyses were performed on a MYECL Imager with MYIMAGEANALYSIS Software. **L** = lysate load; **E1-E5** = eluates from runs 1-5.

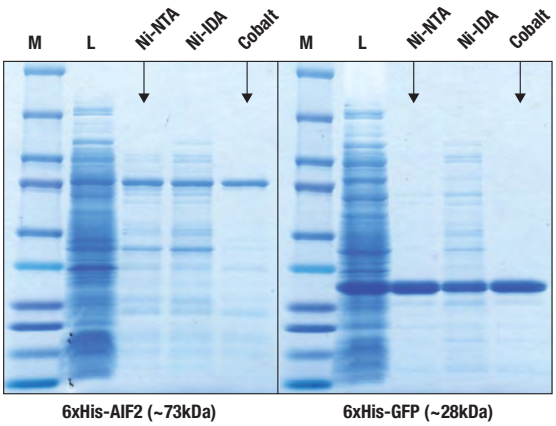



**Figure 4. High-performance purification of different sized proteins using Thermo Scientific HisPur Ni-NTA Agarose Resin.** Bacterial lysate containing over-expressed 6xHis-AIF2 (6mg total protein) or 6xHis-GFP (4mg total protein) was applied to HisPur Ni-NTA Agarose Resin (0.2mL) and purified by the 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. Gels 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.


## Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 88221 | HisPur Ni-NTA Agarose Resin | 10mL |
| 88222 | HisPur Ni-NTA Agarose Resin | 100mL |
| 88223 | HisPur Ni-NTA Agarose Resin | 500mL |
| 88224 | HisPur Ni-NTA Agarose Spin Columns, 0.2mL | 25 columns |
| 88225 | HisPur Ni-NTA Agarose Spin Columns, 1mL | 5 columns |
| 88226 | HisPur Ni-NTA Agarose Spin Columns, 3mL | 5 columns |
| 88227 | HisPur Ni-NTA Agarose Purification Kit, 0.2mL | 25 columns |
| 88228 | HisPur Ni-NTA Agarose Purification Kit, 1mL | 5 columns |
| 88229 | HisPur Ni-NTA Agarose Purification Kit, 3mL | 5 columns |
| 90098 | HisPur Ni-NTA Agarose Chromatography Cartridges, 1mL | 5 cartridges |
| 90099 | HisPur Ni-NTA Agarose Chromatography Cartridges, 5mL | 2 cartridges |
| 88230 | HisPur Ni-NTA Agarose Spin Plates | 2 plates |

# Ni-NTA Superflow Agarose

**The Thermo Scientific™ HisPur™ Ni-NTA Superflow Agarose** is derivatized with a nitrilotriacetic acid (NTA) chelator charged with divalent nickel ($Ni^{2+}$). It is designed for FPLC purification of polyhistidine-tagged protein. HisPur Ni-NTA Superflow Agarose is comprised of highly crosslinked agarose and does not compress at flow rates that are necessary for medium- or large-scale FPLC purifications. This resin exhibits a high dynamic binding capacity across a range of flow rates and is compatible with common clean-in-place procedures for extended use.

## Highlights

- **High dynamic capacity** – binds > 20mg of pure 6xHis-tagged GFP per milliliter of resin at a linear flow rate of 300cm/hr
- **High purity** – > 80% purity when purifying from lysates
- **Versatile** – purify proteins using native and denaturing conditions
- **Robust** – highly crosslinked beads tolerate linear flow rates up to 1,200cm/hr
- **Compatible** – use with a wide variety of chemicals and pH values
- **Cost effective** – competitively priced and can be reused at least 25 times

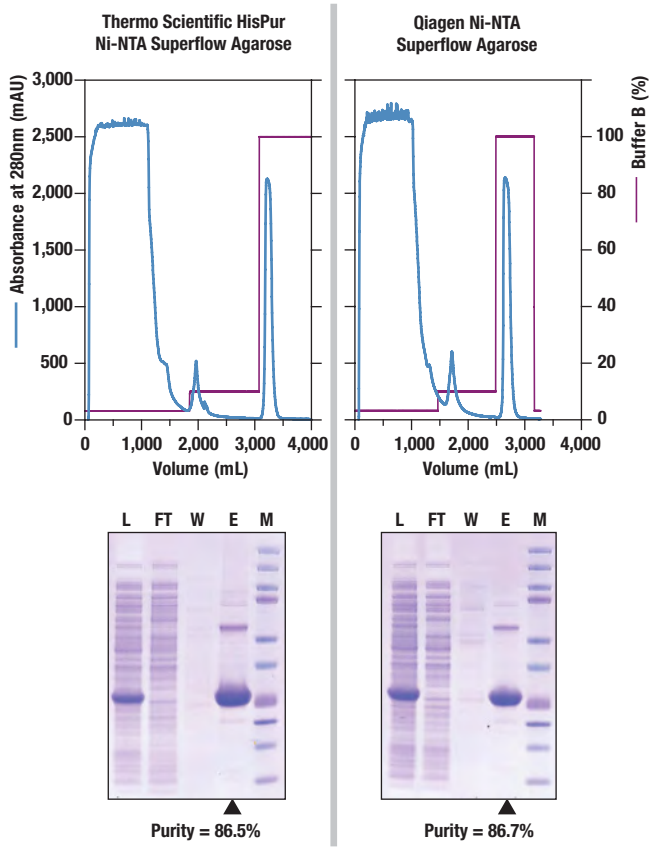
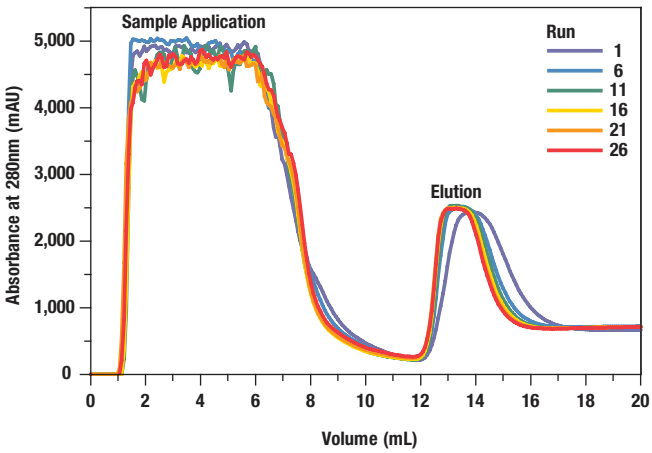



**Figure 1. Thermo Scientific HisPur Ni-NTA Superflow Agarose performs as well as a competitor's resin.** More than 4g of over-expressed 6xHis-GFP were purified in 3 hours using 200mL columns containing HisPur Ni-NTA Superflow Agarose or Qiagen Ni-NTA Superflow. One liter of lysate was loaded at a flow rate of 20mL/min and then washed until baseline with wash buffer containing 30mM imidazole. Bound protein was eluted with buffer containing 300mM imidazole. Protein yields were measured by absorbance at 280nm throughout the FPLC run (chromatogram). Fractions containing purified 6xHis-GFP were pooled and quantitated using the Pierce 660nm Protein Assay (Product # 22662). Load, flow-through, wash and eluate fractions were separated by SDS-PAGE, stained with Imperial Protein Stain (Product # 24615) and evaluated using MVImageAnalysis Software to determine purity. **L** = lysate load; **FT** = flow-through; **W** = wash; **E** = elution; **M** = molecular weight markers.



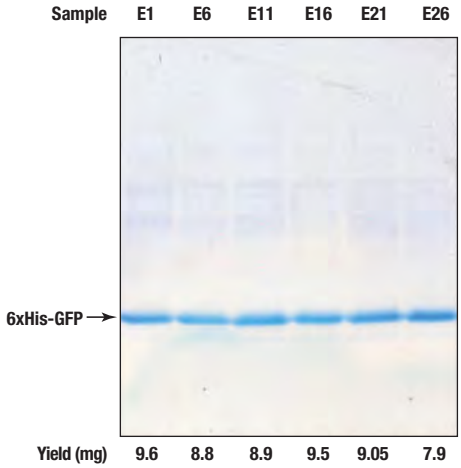




**Figure 2. Dependable reusability of Thermo Scientific HisPur Ni-NTA Superflow Agarose.** 6xHis-GFP lysate (5mL) was loaded onto an equilibrated 1mL column (column diameter = 0.7cm) packed with HisPur Ni-NTA Superflow Agarose at a flow rate of 1mL/min, washed with 5 column volumes (CV) of wash buffer containing 30mM imidazole, and eluted with 10 CV of elution buffer containing 300mM imidazole. The column was washed with 15 CV of 0.5M NaOH at 0.5mL/min to remove nonspecific bound proteins, followed with 10 CV of ultrapure water, and re-equilibration with 10 volumes of binding buffer containing 10mM imidazole. Protein yields were measured in the collected samples by absorbance at 280nm throughout the FPLC run (chromatogram). A lysate challenge was included every fifth cycle for a total of 20 blank runs and six lysate challenges (cycle 1, 6, 11, 16, 21 and 26). 6xHis-GFP yield and purity were similar for all lysate challenges as seen by protein estimation using Pierce 660nm Protein Assay and SDS-PAGE.

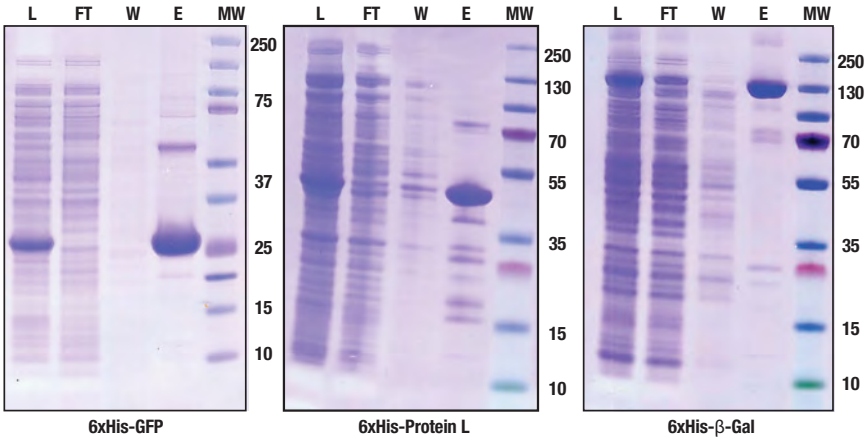



**Figure 3. Efficient purification of three different molecular weight proteins.** 6xHis-GFP (27kDa), 6xHis-Protein L (36kDa) and 6xHis-β-galactosidase (128kDa) were purified from lysates using HisPur Ni-NTA Superflow Agarose. The load, flow-through and eluates were then separated by SDS-PAGE, stained using Imperial Stain for 1 hour and destained with water. Purity analyses were performed on a MYECL Imager with MYIMAGEANALYSIS Software. Greater than 80% purity was observed for 6xHis-GFP and 6xHis β-galactosidase purification. Higher purity for some proteins can be obtained by modifying the imidazole concentrations in the binding and wash buffers. **L** = lysate load; **FT** = flow-through; **W** = wash; **E** = elution; **MW** = molecular weight markers.


## Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 25214 | HisPur Ni-NTA Superflow Agarose | 10mL |
| 25215 | HisPur Ni-NTA Superflow Agarose | 50mL |

## Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 25216 | HisPur Ni-NTA Superflow Agarose | 250mL |
| 25217 | HisPur Ni-NTA Superflow Agarose | 1L |

# resin properties

Table 1. Properties of Thermo Scientific HisPur Ni-NTA Agarose Resin and HisPur Ni-NTA Superflow Agarose.

| | Thermo Scientific HisPur Ni-NTA Agarose Resin | Thermo Scientific HisPur Ni-NTA Superflow Agarose |
|---|---|---|
| **Support** | 6% agarose | 6% agarose, highly crosslinked |
| **Bead Diameter (µm)** | 45-165µm | 60-160µm |
| **Size Exclusion Limit (MW)** | 4 x $10^6$ daltons | 6 x $10^6$ daltons |
| **Maximum Linear Flow Rate**[1] | 800cm/hr | 1,200cm/hr |
| **Recommended Linear Flow Rate** | ≤ 150cm/hr | ≤ 150cm/hr |
| **Maximum Pressure** | 0.35MPa | 0.65MPa |
| **pH Stability (short-term)** | Not determined | 2-10 |
| **pH Stability (long-term)** | Not determined | 3-9 |
| **Antimicrobial Agent** | No | No |
| **Storage** | 20% ethanol | 20% ethanol |
| **Metal Loading** | ≥ 15µmoles $Ni^{+2}$/mL resin | ≥ 15µmoles $Ni^{+2}$/mL resin |
| **Metal Leaching** | ≤ 20ppm | ≤ 20ppm |
| **Static Binding Capacity** | ~60mg of 6xHis-GFP/mL resin | >60mg of 6xHis-GFP/mL resin |
| **Dynamic Binding Capacity**[2] | 18mg of 6xHis-GFP/mL resin | 20mg of 6xHis-GFP/mL resin |
| **Number of Reuses** | 5 | 25 |
| | **Resin Chemical Tolerance**[3] | |
| **Acetic Acid** | Not determined | 1M, (1 week at 37°C) |
| **Sodium Phosphate** | Not determined | 20mM (1 week at 37°C) |
| **SDS** | Not determined | 1% SDS (1 week at 37°C) |
| **Guanidine Hydrochloride** | Not determined | 6M, (1 week at 37°C) |
| **Ethanol** | Not determined | 70%, (1 week at 37°C) |
| **HCl** | Not determined | Not compatible |
| **Urea** | Not determined | 8M, (2 hours at RT) |
| **Glycine** | Not determined | 0.1M, (2 hours at RT) |
| **DTT** | Not determined | 10mM, (2 hours at RT) |
| **β-Mercapatoethanol** | Not determined | Not determined |
| **TCEP** | Not determined | 5mM, (2 hours at RT) |
| **EDTA/EGTA** | Not compatible | Not compatible |


| | Thermo Scientific HisPur Ni-NTA Agarose Resin | Thermo Scientific HisPur Ni-NTA Superflow Agarose |
|---|---|---|
| | **Binding Buffer Chemical Tolerance[4]** | |
| **Wash Buffer (imidazole)** | 20-50mM | 20-50mM |
| **Eultion Buffer (imidazole)** | 200-400mM | 200-400mM |
| **Triton™ X-100** | 2% | 2% |
| **NP-40** | 2% | 2% |
| **SDS** | 0.25% | 0.25% |
| **CHAPS** | 1% | 1% |
| **HEPES** | 100mM | 100mM |
| **Tris** | 100mM | 100mM |
| **Phosphate Buffer** | 50mM | 50mM |
| **MOPS** | 50mM | 50mM |
| **NaCl** | 2M | 2M |
| **β-Mercaptoethanol** | 20mM | 20mM |
| **DTT** | 5mM | 5mM |
| **EDTA** | Not recommended | Not recommended |
| **Guanidinium** | 6M | 6M |
| **Urea** | 8M | 8M |

[1] *Maximum linear flow rate conditions:*
*Column dimensions (w x h): 13mm x 38mm (5mL resin) $dH_2O$ at room temperature*
*Linear flow rate = volumetric flow rate (mL/min) x 60 (min/hr)/cross sectional area ($cm^2$)*

[2] *Dynamic binding conditions (10% breakthrough):*
*Sample: 1mg/mL 6xHis-GFP (27kDa) pure protein in 20mM $NaH_2PO_4$, 300mM NaCl, 10mM imidazole*
*Column dimensions (w x h): 5mm x 50mm (1mL resin)*
*Flow rate: 1mL/min*

[3] *Chemical compatibility:*
*Ni-NTA Agarose resin was incubated in various chemicals for the stated time and at the stated temperature. After incubation, 0.25mL of resin was challenged with 10mg of His-tagged GFP for 30 minutes. The fusion protein was eluted and protein yield measured using the Pierce 660nm Protein Assay. Binding was maintained at > 90% of non-treated control resin.*

[4] *Binding buffer tolerance:*
*Binding buffer tolerances are recommended limits to various chemicals present in the binding buffer. These values were determined by purifying various model proteins in the presence or absence of each chemical and compared to a control sample. Combinations of these chemicals were not tested and could potentially have an additive effect on binding. Each protein is unique and will have different behavior in the presence of these chemicals. Each protein should be empirically tested to determine an optimized purification method.*

# $Co^{2+}$ polyhistidine purification with cobalt

## Cobalt Agarose Resin

**Thermo Scientific™ HisPur™ Cobalt Agarose Resin** is a 6% beaded agarose resin derivatized with a tetradentante chelator charged with divalent cobalt ($Co^{2+}$). HisPur Cobalt Resin provides fast and gentle purification of histidine-tagged fusion proteins with superior purity (up to 97% purity). The agarose resin is ideal for small-scale purification using batch, gravity flow or spin methods. It is also compatible with low flow rate applications on automated FPLC instrumentation.

### Highlights

- **High purity** – obtain >10mg of pure 6xHis-tagged protein per milliliter of resin
- **Specificity** – cobalt-chelate coordination core binds fewer host protein contaminants
- **Low metal leaching** – no metal contamination in eluted histidine-tagged protein sample
- **Versatility** – purify proteins under native or denaturing conditions
- **Flexibility** – available in multiple sizes, including bulk resin, spin columns and chromatography cartridges
- **Cost effective** – resin can be reused five times

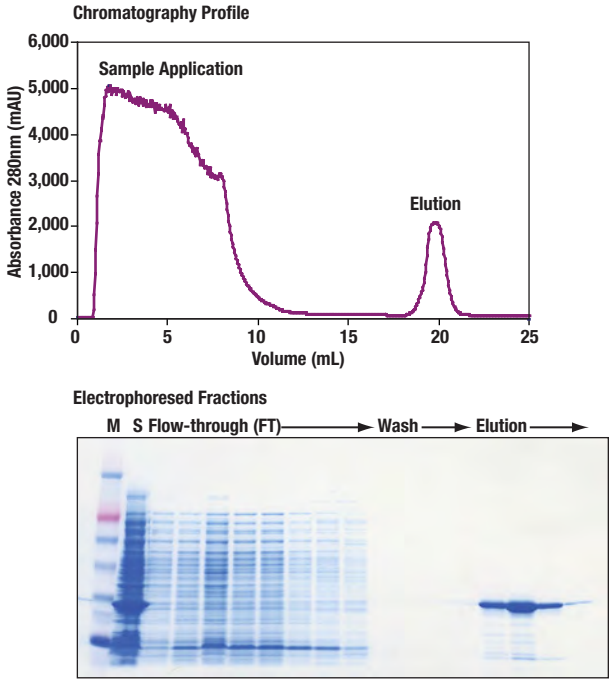



**Figure 1. Purification of 6xHis-GFP from *E. coli* lysate using a Thermo Scientific HisPur Cobalt Agarose Cartridge.** His-tagged GFP was extracted from *E. coli* using Thermo Scientific™ B-PER™ Bacterial Protein Extraction Reagent in Phosphate Buffer (Product # 78266) containing Thermo Scientific™ Halt™ Protease Inhibitor Cocktail, EDTA-Free (Product # 78415). The lysate was diluted 1:1 with equilibration/wash buffer (50mM sodium phosphate, 300mM sodium chloride, 10mM imidazole, pH 7.4) and applied to a HisPur Cobalt Agarose Chromatography Cartridge at a flow rate of 0.3mL/min. The cartridge was washed with equilibration/wash buffer until the baseline absorbance at 280nm was reached. His-tagged GFP was eluted (50mM sodium phosphate, 300mM sodium chloride, 150mM imidazole; pH 7.4). Protein yields were measured in the collected samples by absorbance at 280nm throughout the FPLC run (chromatogram). Selected fractions were analyzed by SDS-PAGE and Thermo Scientific™ GelCode™ Blue Stain Reagent (Product # 24592). Protein yields were measured in the collected samples by absorbance at 280nm throughout the FPLC run (chromatogram). **M** = molecular weight markers; **S** = non-fractionated lysate; **FT** = flow-through.

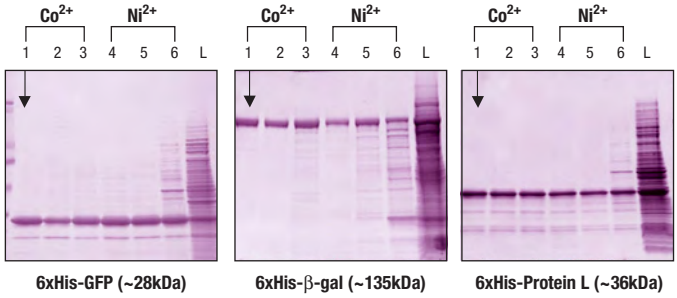



**Figure 2. Thermo Scientific HisPur Cobalt Agarose Resin delivers higher purity than other IMAC resins.** Cell lysates containing over-expressed recombinant 6xHis-tagged protein were prepared in B-PER Bacterial Protein Extraction Reagent (Product # 78243) and Protease Inhibitors (Product # 78410). Protein concentrations were determined by Thermo Scientific™ Coomassie Plus Protein Assay (Product # 23238). *E. coli* lysates containing over-expressed His-tagged GFP, β-galactosidase or Protein L were applied to 0.2mL bed volumes of each IMAC resin in spin column format. Binding, wash and elution buffers were prepared and used according to each manufacturer's instructions. The first elution fraction for each IMAC resin was analyzed by SDS-PAGE and protein purity determined by densitometry. Gel lanes were normalized to equivalent volume. **Lane 1**, HisPur Cobalt Agarose Resin; **Lane 2**, supplier C cobalt resin; **Lane 3**, supplier S cobalt resin; **Lane 4**, supplier G nickel resin; **Lane 5**, supplier Q nickel resin; **Lane 6**, Ni-IDA; and **Lane L**, lysate load. Comparable yield and higher purity were obtained with HisPur Cobalt Agarose Resin (Lane 1) compared to other IMAC resins (Lanes 2-6).

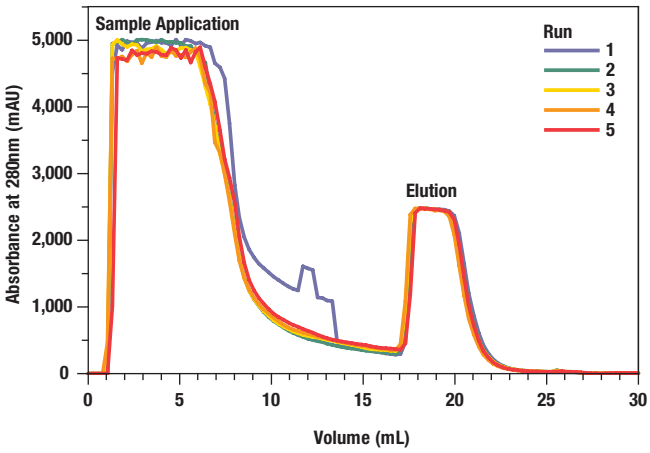
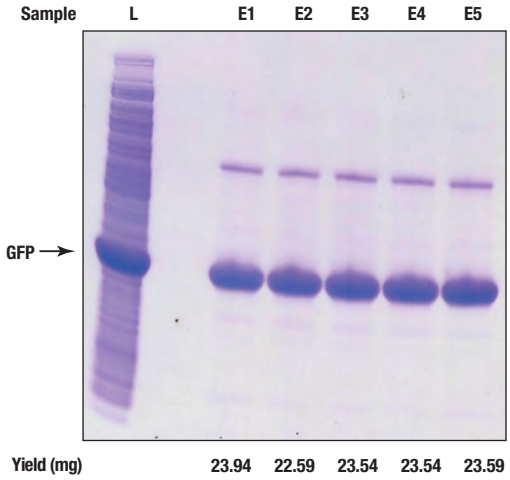



**Figure 3. Reuse Thermo Scientific HisPur Cobalt Agarose Resin at least five times without loss of performance.** Packed HisPur Cobalt Agarose Resin (1mL) was equilibrated with 5 column volumes (CV) of binding buffer (20mM phosphate, 300mM sodium chloride, 5mM imidazole, pH 7.4). GFP lysate (5mL) was injected at 1mL/min onto the column. The column was washed with 10 CV of wash buffer (20mM phosphate, 300mM sodium chloride, 15mM imidazole, pH 7.4). The bound GFP was eluted using 10 CV of elution buffer (20mM phosphate, 300mM sodium chloride, 150mM imidazole, pH 7.4). Protein yields were measured in the collected samples by absorbance at 280nm throughout the FPLC run (chromatogram). The column was regenerated using 10 CV of 20mM MES, 300mM NaCl, pH 5. The column was regenerated and reused five times. The amount of protein in each elution was determined using the Pierce 660nm Protein Assay and 5µg of eluate (E) were loaded on the gel. The gel was stained with Imperial Protein Stain for one hour and destained with water. Purity analyses were performed on a MYECL Imager with MYIMAGEANALYSIS Software. **L** = lysate load; **E1-E5** = eluates from runs 1-5.




## Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 89964 | HisPur Cobalt Agarose Resin | 10mL |
| 89965 | HisPur Cobalt Agarose Resin | 100mL |
| 89966 | HisPur Cobalt Agarose Resin | 500mL |
| 89967 | HisPur Cobalt Agarose Spin Columns, 0.2mL | 25 columns |
| 89968 | HisPur Cobalt Agarose Spin Columns, 1mL | 5 columns |
| 89969 | HisPur Cobalt Agarose Spin Columns, 3mL | 5 columns |
| 90090 | HisPur Cobalt Agarose Purification Kit, 0.2mL | 25 columns |
| 90091 | HisPur Cobalt Agarose Purification Kit, 1mL | 5 columns |
| 90092 | HisPur Cobalt Agarose Purification Kit, 3mL | 5 columns |
| 90093 | HisPur Cobalt Agarose Chromatography Cartridges, 1mL | 5 cartridges |
| 90094 | HisPur Cobalt Agarose Chromatography Cartridges, 5mL | 2 cartridges |
| 90095 | HisPur Cobalt Agarose Spin Plates | 2 plates |

# $Co^{2+}$ polyhistidine purification with cobalt

## Cobalt Superflow Agarose

**The Thermo Scientific™ HisPur™ Cobalt Superflow Agarose** is derivatized with a tetradentate chelator charged with divalent cobalt ($Co^{2+}$). It is designed for high-purity, large-scale purifications of polyhistidine-tagged protein. HisPur Cobalt Resin is superior to traditional nickel-based immobilized metal affinity chromatography (IMAC) systems. Compared with nickel-based IMAC resins, HisPur Cobalt Resin binds histidine-tagged proteins with higher specificity (less off-target binding), displays less metal leaching and enables less stringent elution conditions resulting in higher purity.

### Highlights

- **High yields** – binds 20-30mg of 6xHis-GFP per milliliter of resin at a linear flow rate of 150cm/hr
- **Robust** – tolerates linear flow rates up to 1,200cm/hr with minimal leaching of cobalt due to a tetradentate chelator
- **High purity** – specificity of cobalt binding to histidine-tag generally results in elution fractions with less than 10% contamination of nonspecific proteins
- **Compatible** – maintains function after exposure to a wide variety of chemicals and pH values
- **Cost effective** – resin can be reused > 25 times with no loss in performance

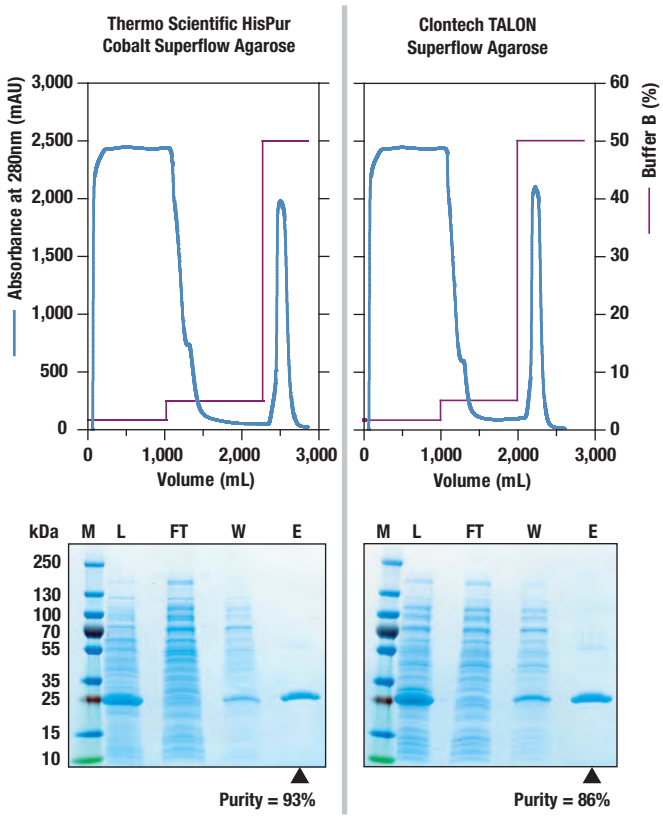
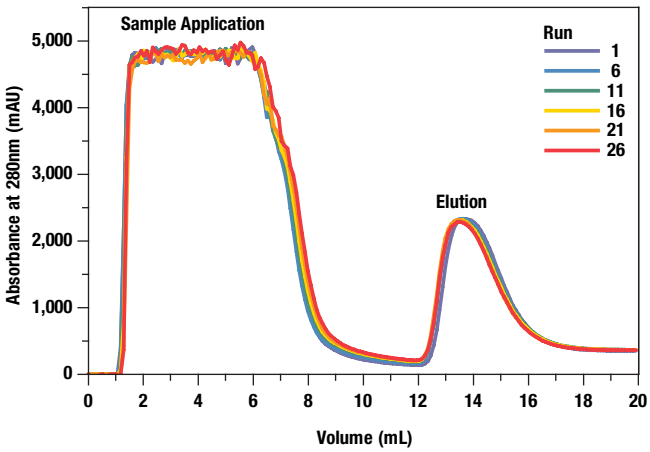



**Figure 1. Medium-scale FPLC purification of 6xHis-GFP produces > 90% purity of target protein.** Biomass (100g) containing over-expressed 6xHis-GFP was lysed with 1L of lysis buffer supplemented with Halt Protease Inhibitor (Product # 78439) and loaded onto an equilibrated 200mL column (50mm x ~100mm) of HisPur Cobalt Superflow Agarose or TALON™ Superflow at a flow rate of 20mL/min. The columns were then washed until baseline with wash buffer containing 15mM imidazole. Bound protein was eluted with buffer containing 150mM imidazole. Protein yields were measured in the collected samples by absorbance at 280nm throughout the FPLC run (chromatogram). Fractions containing purified 6xHis-GFP were pooled and quantitated using the Pierce 660nm Protein Assay (Product # 22662). Load, flow-through, wash and eluate fractions were separated by SDS-PAGE to determine purity. Total yield, recovery and purity were similar for both resins. **M** = molecular weight markers; **L** = lysate load; **FT** = flow-through; **W** = wash; **E** = elution.



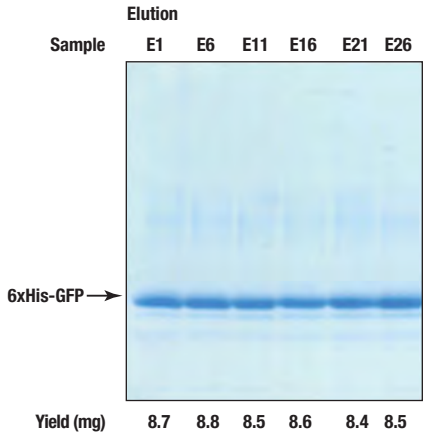




**Figure 2. High reusability of Thermo Scientific HisPur Cobalt Superflow Agarose.** 6xHis-GFP lysate (5mL) was loaded onto an equilibrated 1mL column (column diameter = 0.7cm) packed with HisPur Cobalt Superflow Agarose at a flow rate of 1mL/min, washed with 5 column volumes (CV) of wash buffer containing 15mM imidazole, and eluted with 10 CV of elution buffer containing 150mM imidazole. Protein yields were measured in the collected samples by absorbance at 280nm throughout the FPLC run (chromatogram). The column was regenerated with 10 CV of MES buffered saline (20mM MES pH 5.0, 300mM NaCl) to remove imidazole, and then 10 CV of ultrapure water, followed by equilibration with 10 volumes of binding buffer containing 5mM 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 Pierce 660nm Protein Assay and SDS-PAGE.

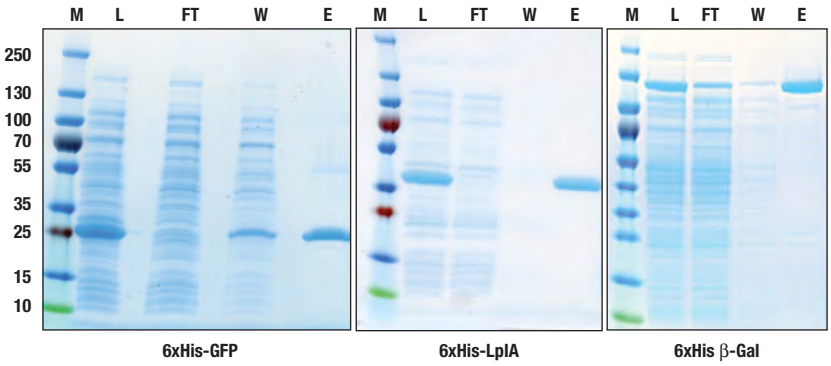



**Figure 3. Robust purification of three different molecular weight proteins using Thermo Scientific HisPur Cobalt Superflow Agarose.** *E. coli* over-expression lysates of GFP, LplA or β-gal fusion protein (40mL total) were generated by resuspending pellets in 10 volumes of lysis buffer and passing through a microfluidizer twice. Lysates were cleared by centrifugation and then loaded onto columns packed with 10mL of HisPur Cobalt Superflow Agarose. The columns were washed with 5 column volumes of buffer containing 10mM imidazole. Bound protein was then eluted with buffer containing 150mM imidazole, and fractions containing protein were pooled. Each fraction was separated by SDS-PAGE and stained with GelCode Blue Stain Reagent (Product # 24590). **L** = load; **FT**= flow-through; **W** = wash; and **E** = eluate.


## Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 25228 | HisPur Cobalt Superflow Agarose | 10mL |
| 25229 | HisPur Cobalt Superflow Agarose | 50mL |
| 25230 | HisPur Cobalt Superflow Agarose | 250mL |
| 25231 | HisPur Cobalt Superflow Agarose | 1L |

# resin properties

Table 1. Properties of Thermo Scientific HisPur Cobalt Agarose Resin and Thermo Scientific HisPur Cobalt Superflow Agarose.

| | Thermo Scientific HisPur Cobalt Agarose Resin | Thermo Scientific HisPur Cobalt Superflow Agarose |
|---|---|---|
| **Support** | 6% agarose | 6% agarose, highly crosslinked |
| **Bead Diameter (µm)** | 45-165µm | 60-160µm |
| **Size Exclusion Limit (MW)** | 4 x $10^6$ daltons | 6 x $10^6$ daltons |
| **Maximum Linear Flow Rate**[1] | 800cm/hr | 1,200cm/hr |
| **Recommended Linear Flow Rate** | ≤ 150cm/hr | ≤ 150cm/hr |
| **Maximum Pressure** | 0.35MPa | 0.65MPa |
| **pH Stability (short term)** | Not determined | 2-12 |
| **pH Stability (long term)** | Not determined | 3-9 |
| **Antimicrobial Agent** | None | None |
| **Storage** | 4°C in 20% Ethanol | 4°C in 20% Ethanol |
| **Metal Loading** | > 12µmol $Co^{2+}$/mL | > 11µmol $Co^{2+}$/mL |
| **Metal Leaching** | ≤ 0.2ppm | ≤ .050ppm |
| **Static Binding Capacity** | ≥ 15mg 6xHis-GFP/mL resin | > 30mg 6xHis-GFP/mL resin |
| **Dynamic Binding Capacity**[2] | Not determined | > 20mg 6xHis-GFP/mL resin |
| **Number of Reuses** | Up to 5 times | Up to 25 times |
| | **Resin Chemical Tolerance**[3] | |
| **Acetic Acid** | Not determined | 1M, pH 2 (1 week at 37°C) |
| **Sodium Phosphate** | Not determined | 20mm |
| **SDS** | Not determined | 1% at pH 7 (1 week at 37°C) |
| **Guanidine Hydrochloride** | Not determined | 6M (1 week at 37°C) |
| **Urea** | Not determined | 8M (2 hours at RT) |
| **Ethanol** | Not determined | 70% (1 week at 37°C) |
| **0.1M HCl** | Not determined | Not compatible |
| **Glycine** | Not determined | 0.1M, pH 12 |
| **DTT** | Not determined | 10mm (2 hours at RT) |
| **β-Mercapatoethanol** | Not determined | 10mm (2 hours at RT) |
| **TCEP** | Not determined | 5mM (2 hours at RT) |
| **EDTA/EGTA** | Not determined | Not compatible |


| | Thermo Scientific HisPur Cobalt Agarose Resin | Thermo Scientific HisPur Cobalt Superflow Agarose |
|---|---|---|
| **Binding Buffer Chemical Tolerance[4]** | | |
| **Wash Buffer (imidazole)** | 5-15mM | 5-15mM |
| **Eultion Buffer (imidazole)** | 100-250mM | 100-250mM |
| **Triton X-100** | 1% | 1% |
| **NP-40** | 1% | 1% |
| **SDS** | 0.25% | 0.25% |
| **CHAPS** | 1% | 1% |
| **HEPES** | 100mM | 100mM |
| **Tris** | 100mM | 100mM |
| **Phosphate Buffer** | 50mM | 50mM |
| **MOPS** | 50mM | 50mM |
| **NaCl** | 1M | 1M |
| **β-Mercaptoethanol** | 10mM | 10mM |
| **DTT** | 5mM | 5mM |
| **EDTA** | Not recommended | Not recommended |
| **Guanidinium** | 6M | 6M |
| **Urea** | 8M | 8M |

[1] *Max linear flow rate conditions*
*Column dimensions (w x h): 13mm x 38mm (5mL resin) $dH_2O$ at room temperature*
*Linear flow rate = volumetric flow rate (mL/min) x 60 (min/hr)/cross sectional area ($cm^2$)*

[2] *Dynamic binding conditions (10% breakthrough):*
*Sample: 1mg/mL 6xHis-GFP (27kDa) pure protein in 20mM $NaH_2PO_4$, 300mM NaCl, 5mM imidazole*
*Column dimensions (w x h): 5mm x 50mm (1mL resin)*
*Flow rate: 1mL/min*

[3] *Chemical compatibility:*
*Cobalt resin was incubated in various chemicals for the stated time and at the stated temperature. After incubation, 0.25mL of resin was challenged with 5mg of His-tagged GFP for 30 minutes. The fusion protein was eluted and protein yield measured using the Pierce 660nm Protein Assay. Binding was maintained at >90% of non-treated control resin.*

[4] *Binding buffer tolerance:*
*Binding buffer tolerances are recommended limits to various chemicals present in the binding buffer. These values were determined by purifying various model proteins in the presence or absence of each chemical and compared to a control sample. Combinations of these chemicals were not tested and could potentially have an additive effect on binding. Each protein is unique and will have different behavior in the presence of these chemicals. Each protein should be empirically tested to determine an optimized purification method.*

# Glutathione Magnetic Beads

**The Thermo Scientific™ Pierce™ Glutathione Magnetic Beads** provide a simple, rapid and reliable method for the purification of glutathione S-transferase (GST) fusion proteins from crude cell lysates prepared from bacteria, yeast or mammalian cells. These beads can be used to isolate GST-tagged proteins or perform pull-down assays using GST-tagged proteins as bait.

## Highlights

- **High binding** – binds 5-10mg GST/mL settled beads
- **Stable-affinity ligand** – glutathione is covalently immobilized to particles, ensuring leach-resistance and clean purification products
- **High capacity** – binding capacity is sufficient for both routine and demanding magnetic separation procedures

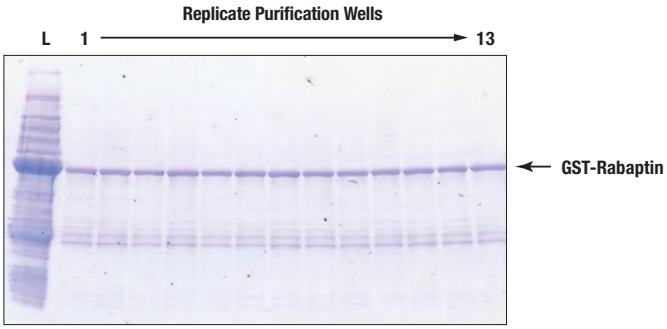



**Figure 1. High-performance purification of a GST fusion protein using Thermo Scientific Pierce Glutathione Magnetic Beads.** Bacterial cells expressing GST-Rabaptin were lysed, and replicate aliquots were processed with the Glutathione Magnetic Beads in a 96 deep-well plate using a Thermo Scientific™ KingFisher™ 96 Instrument. Eluates were boiled in reducing sample buffer, resolved by SDS-PAGE and stained with coomassie dye. Purity and reproducibility were excellent. **L** = lysate load.


## Ordering Information

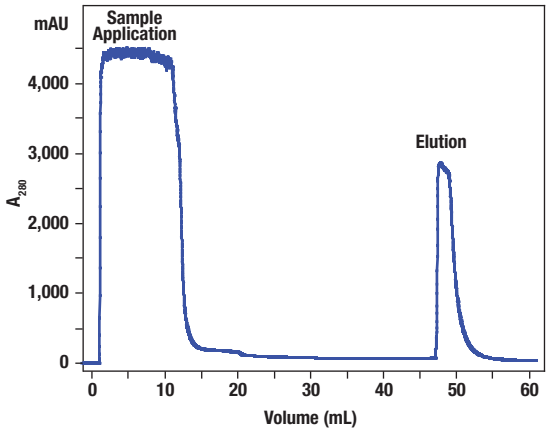
| Product # | Description | Pkg. Size |
|---|---|---|
| 88821 | Pierce Glutathione Magnetic Beads<br>*Sufficient for: Binding 5 to 10mg GST per mL of beads* | 4mL |
| 88822 | Pierce Glutathione Magnetic Beads<br>*Sufficient for: Binding 5 to 10mg GST per mL of beads* | 20mL |

# Glutathione Agarose

**Thermo Scientific™ Pierce™ Glutathione Agarose** consists of reduced glutathione that has been immobilized via a 12-atom spacer arm to crosslinked 6% beaded agarose. This resin selectively binds GST fusion proteins from cell lysates for purification. Pierce Glutathione Agarose can also be used to perform GST-fusion protein pull-down assays. The agarose resin is ideal for small-scale purification using batch, gravity flow or spin methods. It is also compatible with low flow rate applications on automated FPLC instrumentation.

### Highlights

- **High capacity** – binds at least 40mg of recombinant GST protein per milliliter of resin
- **High yield and purity** – consistently purifies at least 10mg of GST-tagged protein per milliliter of resin with greater than 90% purity
- **Cost effective** – resin can be reused at least five times without reduction in binding capacity and purification performance
- **Versatile** – works well to purify GST-fusion proteins from bacterial lysates or use with pre-purified GST-tagged proteins to pull down protein interactions
- **Compatible** – validated and effective for use with Thermo Scientific Cell Lysis Reagents to extract and purify from bacterial or mammalian cell cultures
- **Flexible** – available in multiple formats, including bulk resin, spin columns, chromatography cartridges and convenient GST purification kits



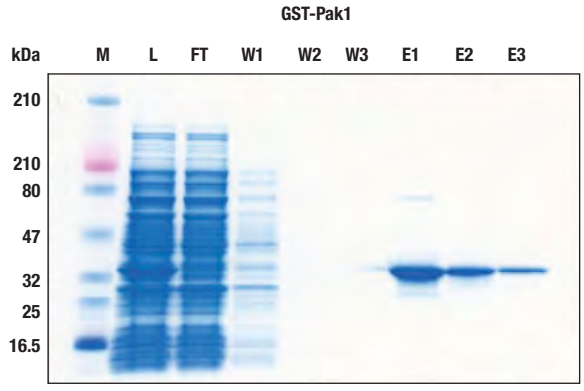
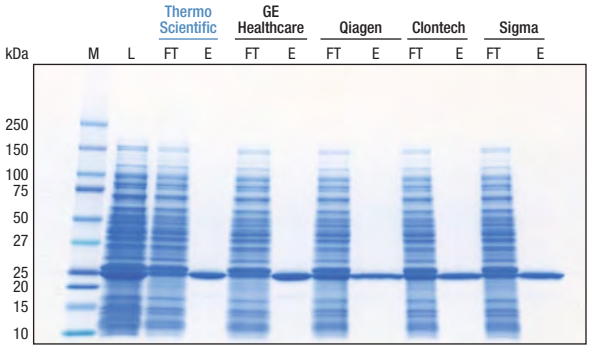



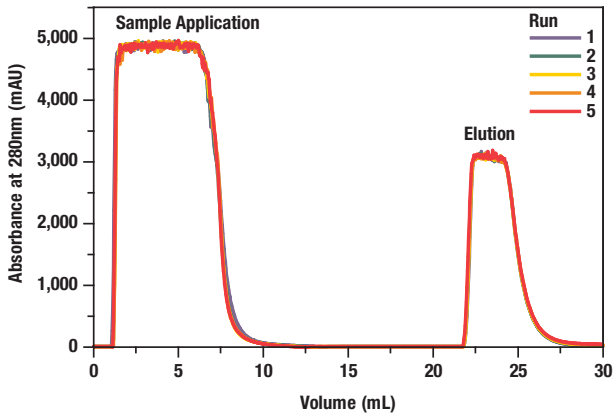

**Figure 1. High-quality purification of Pak 1-GST fusion protein using Thermo Scientific Pierce Glutathione Agarose.** A Pierce GST Spin Purification Kit (Product # 16106) was used to purify Pak 1-GST fusion protein. Pak 1-GST lysate (2.4mg total protein) was applied in Glutathione Binding Buffer to a 0.2mL Pierce Glutathione Spin Column and eluted with 10mM Glutathione Elution Buffer, pH 8.0. Protein yields were measured in the collected samples by absorbance at 280nm throughout the FPLC run (chromatogram). Fractions were resolved by SDS-PAGE using a 4-20% Tris-Glycine gel. The gel was stained with GelCode Blue Stain Reagent (Product # 24590). Thermo Scientific™ Pierce™ 3-Color Protein Molecular Weight Marker Mix (Product # 26691) was used. **M**= molecular weight markers; **L** = lysate load; **FT** = flow-through; **W** = washes; **E** = elutions.

# Glutathione Agarose (continued)




| Vendor | Thermo Scientific | GE Healthcare | Qiagen | Clontech | Sigma |
|---|---|---|---|---|---|
| Yield | 537µg | 562µg | 285µg | 299µg | 410µg |
| Purity | 93% | 93% | 90% | 91% | 94% |

**Figure 2. Thermo Scientific Pierce Glutathione Agarose delivers high-purity GST-fusion proteins with high yields.** *E. coli* lysate (14.4mg total protein) containing an over-expressed GST-fusion protein was incubated with 50µL GSH resin from various suppliers and purified per manufacturer's instructions. The amount of GST eluted from the resin (yield) was quantified by Coomassie Plus Protein Assay. Purity was assessed by densitometry of the stained gel lanes. **M** = molecular weight markers; **L** = lysate load; **FT** = flow-through; **E** = elution.



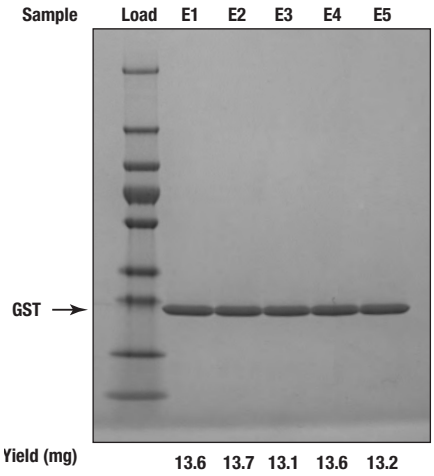




**Figure 3. Thermo Scientific Pierce Glutathione Agarose can be used multiple times without loss of resolution or yield.** Biomass containing over-expressed GST was lysed in lysis buffer at a ratio of 10mL/1g of biomass and 5.0mL was loaded onto a 1mL Pierce Glutathione Agarose Cartridge (Product # 16109) at a linear flow rate of 77cm/hr. The column was washed with 15 column volumes of wash buffer and the GST protein was eluted with reduced glutathione. Protein yields were measured in the collected samples by absorbance at 280nm throughout the FPLC run (chromatogram). The column was then regenerated by passing 25mL of wash buffer over the column. This cycle was repeated four times for a total of five purifications with the same Pierce Glutathione Agarose Resin. The eluted fractions were separated by SDS-PAGE and stained with Imperial Protein Stain (Product # 24615). The FPLC $A_{280}$ profile, protein yield and purity were consistent across all five reuses.

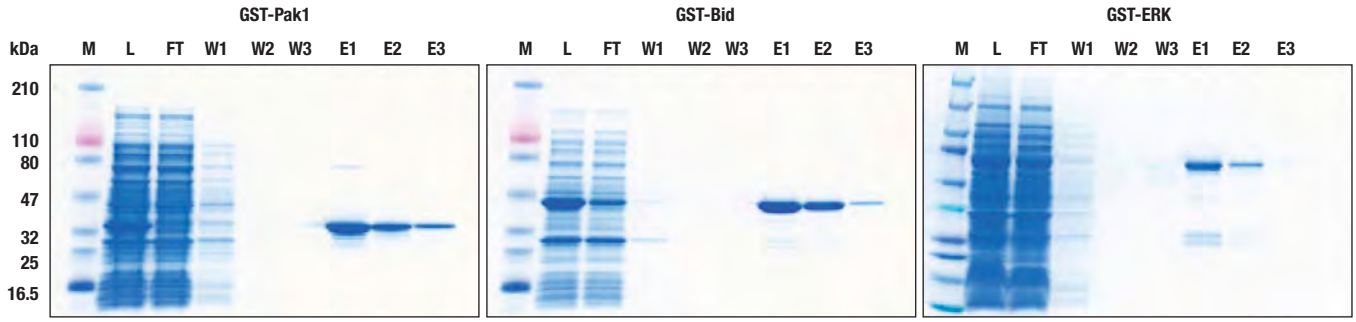



**Figure 4. High-quality purification of different GST-fusion proteins using Thermo Scientific Pierce Glutathione Agarose.** Three GST-fusion proteins were expressed in *E. coli*, extracted in B-PER Reagent with Enzymes (Product # 90078) and then purified using Pierce Glutathione Agarose. Elution fractions were separated on 4-20% gradient Tris-glycine gels and stained with GelCode Blue Stain Reagent (Product # 24590). **M** = molecular weight markers; **L** = lysate load; **FT** = flow-through; **W** = wash; **E** = elution.


## Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 16100 | Pierce Glutathione Agarose | 10mL |
| 16101 | Pierce Glutathione Agarose | 100mL |
| 16102 | Pierce Glutathione Agarose | 500mL |
| 16103 | Pierce Glutathione Spin Columns, 0.2mL | 25 columns |
| 16104 | Pierce Glutathione Spin Columns, 1mL | 5 columns |
| 16105 | Pierce Glutathione Spin Columns, 3mL | 5 columns |
| 16106 | Pierce GST Spin Purification Kit, 0.2mL | 25 column kit |
| 16107 | Pierce GST Spin Purification Kit, 1mL | 5 column kit |
| 16108 | Pierce GST Spin Purification Kit, 3mL | 5 column kit |
| 16109 | Pierce Glutathione Chromatography Cartridges, 1mL | 5 cartridges |
| 16110 | Pierce Glutathione Chromatography Cartridges, 5mL | 2 cartridges |
| 16111 | Pierce Glutathione Spin Plates | 2 plates |

# Glutathione Superflow Agarose

**Thermo Scientific™ Pierce™ Glutathione Superflow Agarose** consists of glutathione that has been immobilized to a crosslinked 6% beaded agarose that is designed for high-purity, large-scale purification of GST-tagged proteins. Pierce Glutathione Superflow Agarose is a highly crosslinked, durable resin that does not compress at flow rates necessary for medium- to large-scale FPLC purifications. The resin holds up well to a variety of chemical and pH values, and it is compatible with common clean-in-place procedures. The specific binding of glutathione to the GST-tag results in high purity and high yield in a single-step purification.

## Highlights

- **High dynamic binding capacity** – binds 10-20mg of pure GST per milliliter of resin at linear flow rates ranging between 150-30cm/hr, respectively
- **High purity** – gives > 90% purity of eluted fractions when used to purify from lysates
- **Robust** – highly crosslinked beads tolerates linear flow rates up to 1,200cm/hr
- **Compatible** – use with a wide variety of chemicals and pH values
- **Cost effective** – reusable at least 25 times

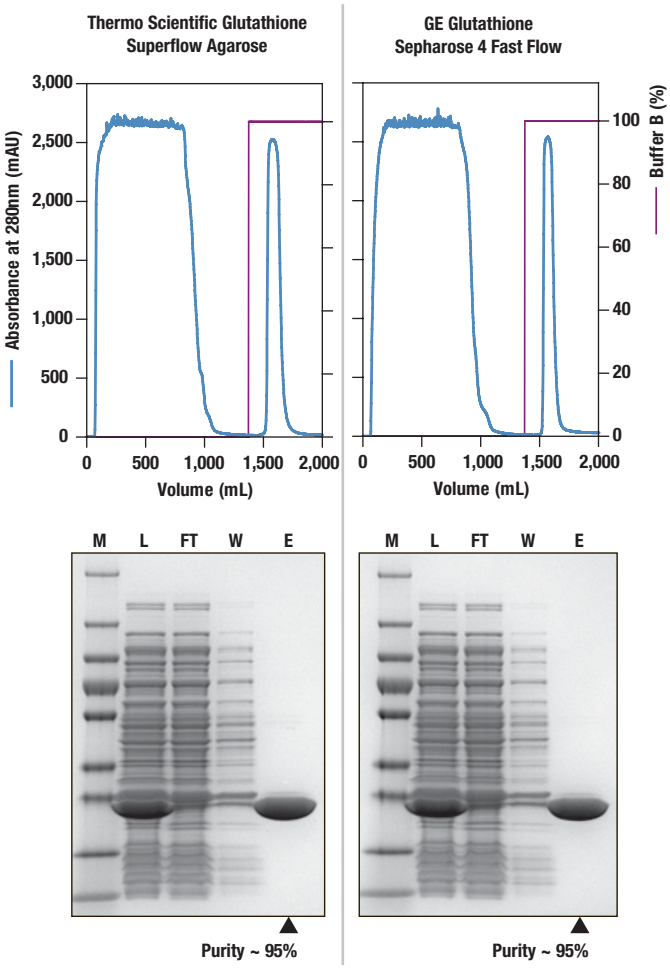
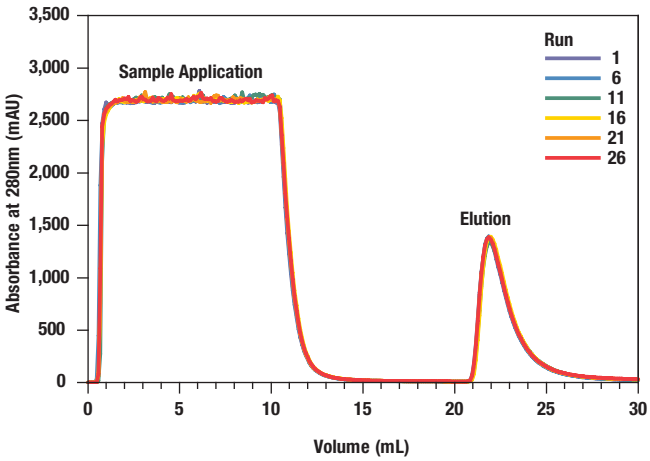



**Figure 1. Large-scale FPLC purification of GST produces > 95% purity of target protein.** Biomass (170g) containing over-expressed GST was lysed with 1.7L of lysis buffer and then 0.75L of lysate was loaded onto equilibrated 200mL columns (50mm x 100mm) of Thermo Scientific Pierce Glutathione Superflow Agarose (left) or GE Glutathione Sepharose 4 Fast Flow (right) at a linear flow rate of 30cm/hour. Columns were washed with binding buffer until the $UV_{280}$ reached baseline; then bound protein was eluted with elution buffer and fractions containing purified GST were pooled. Protein yields were measured in the collected samples by absorbance at 280nm throughout the FPLC run (chromatogram). Load, flow-through, wash and eluate fractions were separated by SDS-PAGE, stained with Imperial Protein Stain (Product # 24615) and evaluated using MyImageAnalysis Software to determine purity. Total yield, recovery, and purity were nearly identical for both resins. **M** = molecular weight markers; **L** = lysate load; **FT** = flow-through; **W** = wash; **E** = elution.



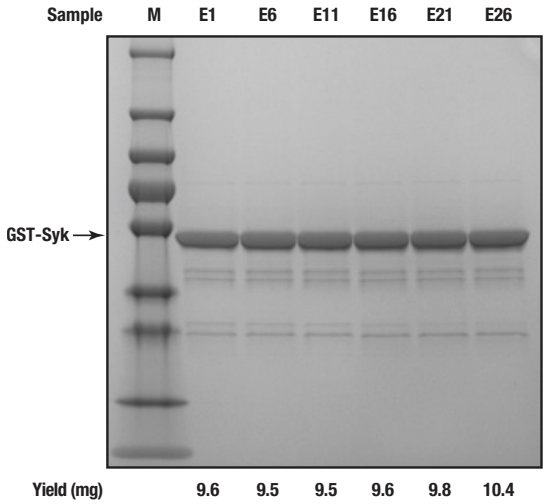




**Figure 2. Dependable reusability of Thermo Scientific Pierce Glutathione Superflow Agarose.** Glutathione Superflow Agarose was challenged with multiple rounds of protein purification and column cleaning. An equilibrated 1mL column (column diameter = 0.5cm) packed with Glutathione Superflow Agarose and attached to a GE AKTA FPLC was challenged with 10mL of *E. coli* lysate contain over-expressed GST-Syk at a flow rate of 0.5mL/min. After loading GST-Syk onto the column, it was washed with 10 column volumes (CV) of wash buffer followed by 10 CV of elution buffer containing 10mM reduced glutathione. After GST-Syk protein elution, the column was treated to 5 clean-in-place cycles. One clean-in-place cycle consists of treating the column to 2 CV of 6M Guanidine-HCl, 5 CV of wash buffer, 4 CV of 70% ethanol followed with 5 CV of wash buffer. Purification followed by 5 clean-in-place cycles was repeated 5 times, for a total of 6 lysate challenges (cycle 1, 6, 11, 16, 21, and 26) and 25 clean-in-place treatments. GST protein yield and purity were measured by absorbance at 280nm and the chromatogram was depicted for each of the 5 lysate challenges. Elution fractions were also analyzed by SDS-PAGE, which also revealed pure, consistent GST-Syk. **M** = molecular weight markers.

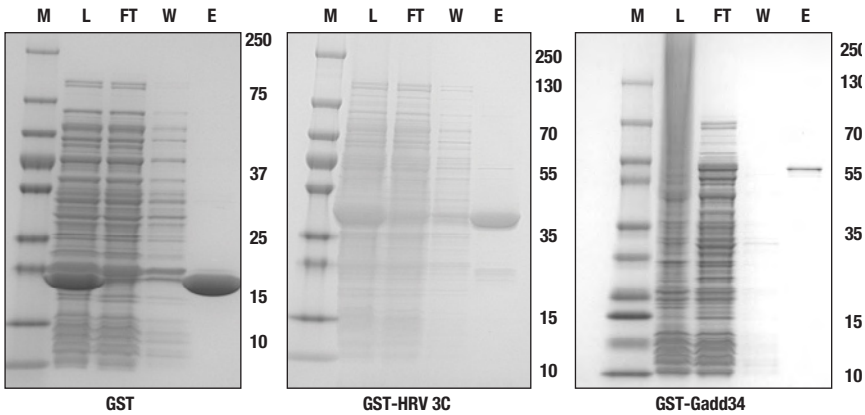



**Figure 3. Efficient purification of three different molecular weight proteins using Thermo Scientific Pierce Glutathione Superflow Agarose.** Three different GST-fusion proteins were purified from lysates using Pierce Glutathione Superflow Agarose. The load, flow-through and eluates were then separated by SDS-PAGE and stained using Imperial Stain (Product # 26415). **M** = molecular weight markers; **L** = lysate load; **FT** = flow-through; **W** = wash; **E** = elution.


## Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 25236 | Pierce Glutathione Superflow Agarose | 10mL |
| 25237 | Pierce Glutathione Superflow Agarose | 50mL |
| 25238 | Pierce Glutathione Superflow Agarose | 250mL |
| 25239 | Pierce Glutathione Superflow Agarose | 1L |

# resin properties

Table 1. Properties of Thermo Scientific Pierce Glutathione Agarose and Thermo Scientific Pierce Glutathione Superflow Agarose.

| | Thermo Scientific Glutathione Agarose | Thermo Scientific Glutathione Superflow Agarose |
|---|---|---|
| **Support** | 6% agarose | 6% agarose, highly crosslinked |
| **Bead Diameter (µm)** | 45-165µm | 60-160µm |
| **Size Exclusion Limit (MW)** | $4 \times 10^6$ | $6 \times 10^6$ |
| **Maximum Linear Flow Rate**[1] | 800cm/hr | 1,200cm/hr |
| **Recommended Linear Flow Rate** | ≤ 150cm/hr | ≤ 150cm/hr |
| **Maximum Pressure** | 0.35MPa | 0.65MPa |
| **pH Stability (short term)** | Not determined | 3-9 (≥ 1 week at 4°C) |
| **pH Stability (long term)** | 4-13 | 2-12 (≥ 2hr at 22°C) |
| **Antimicrobial Agent** | 0.05% sodium azide | 0.05% sodium azide |
| **Storage** | 4°C | 4°C |
| **Static Binding Capacity** | ~ 40mg of GST | ~ 30mg of GST |
| **Dynamic Binding Capacity**[2] | ~ 10.5mg of GST | ~ 10mg of GST |
| **Number of Reuses** | 5 | 25 |
| | **Chemical Compatibility**[3] | |
| **Acetic acid** | Not determined | 1M (1 week at 37°C) |
| **SDS** | Not determined | 1% (1 week at 37°C) |
| **Guanidine Hydrochloride** | Not determined | 6M (1 week at 37°C) |
| **Ethanol** | Not determined | 70% (1 week at 37°C) |
| **HCl** | Not determined | 0.1M, pH 1 (2hr at 22°C) |
| **Urea** | Not determined | 8M (2hr at 22°C) |
| **NaOH** | Not determined | 0.1M (2hr at 22°C) |

| | Thermo Scientific Glutathione Agarose | Thermo Scientific Glutathione Superflow Agarose |
|---|---|---|
| **Binding Buffer Chemical Tolerances[4]** | | |
| **Eultion Buffer (reduced glutathione)** | 5-50mM | 5-50mM |
| **Triton X-100** | 1% | 1% |
| **NP-40** | 1% | 1% |
| **SDS** | Not compatible | Not compatible |
| **CHAPS** | Not compatible | Not compatible |
| **HEPES** | 100mM | 100mM |
| **Tris** | 100mM | 100mM |
| **Phosphate Buffer** | 50mM | 50mM |
| **MOPS** | 50mM | 50mM |
| **NaCl** | 1M | 1M |
| **β-Mercaptoethanol** | 10mM | 10mM |
| **DTT** | 4mM | 4mM |
| **EDTA** | 10mM | 10mM |
| **Guanidinium** | Not compatible | Not compatible |
| **Urea** | Not compatible | Not compatible |

[1] *Max linear flow rate conditions:*
*Column dimensions (w x h): 13mm x 38mm (5mL resin)*
*Ultrapure water at room temperature*
*Linear flow rate = (volumetric flow rate (mL/min) x 60 (min/hr))/cross sectional area ($cm^2$)*

[2] *Dynamic binding conditions (10% breakthrough):*
*Sample: 1mg/mL GST (26kDa) pure protein in 50mM Tris, pH 8.0, 150mM NaCl*
*Column dimensions (w x h): 5mm x 50mm (1mL resin)*
*Flow rate: 0.5mL/min*

[3] *Chemical compatibility:*
*Glutathione resin was incubated in various chemicals for the stated time and at the stated temperature. After incubation, 50µL of resin was challenged with 2.5mg GST for 2 hours. The fusion protein was eluted and protein yield measured using the Pierce 660nm Protein Assay. Binding was maintained at > 90% of non-treated control resin.*

[4] *Binding buffer tolerance:*
*Binding buffer tolerances are recommended limits to various chemicals present in the binding buffer. These values were determined by purifying various model proteins in the presence or absence of each chemical and compared to a control sample. Combinations of these chemicals were not tested and could potentially have an additive effect on binding. Each protein is unique and will have different behavior in the presence of these chemicals. Each protein should be empirically tested to determine an optimized purification method.*

# HRV 3C Protease

**The Thermo Scientific™ Pierce™ HRV 3C Protease** is a highly specific cysteine protease that can be used to selectively cleave affinity tags (6xHis or GST) from fusion proteins. HRV 3C Protease is dual tagged for easy removal after cleavage and is highly specific for the recognition sequence Leu-Glu-Val-Leu-Phe-Gln-↓-Gly-Pro, cleaving after the glutamine. Cloning this cleavage sequence between your protein of interest and the affinity tag enables sight-selective cleavage. Pierce HRV 3C Protease can be used for on-column cleavage or for easy affinity tag removal in solution.

## Highlights

- **High activity at 4°C** – activity is ≥ 2,000U/mg of a control protein; one unit will cleave 0.1mg of a control protein at 4°C after 16 hours
- **Compatible** – active in a wide range of buffers
- **Flexible** – perform on-column or in-solution cleavage

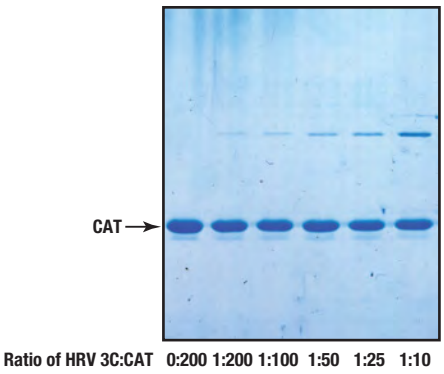



**Figure 1. Thermo Scientific Pierce HRV 3C Protease selectively cleaves only at the correct amino acid sequence.** HRV 3C Protease (1µg) was incubated with increasing amounts of chloramphenicol acetyl transferase (CAT), which does not contain the HRV 3C cleavage site. After a 16-hour incubation at 4°C, 1µg of CAT was analyzed by SDS-PAGE. The presence of cleavage products was not detected.

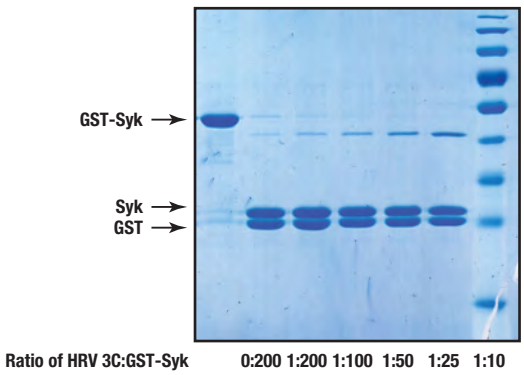



**Figure 2. Thermo Scientific Pierce HRV 3C Protease efficiently cleaves at a wide variety of enzyme:substrate ratios.** HRV 3C Protease (1µg) was incubated with increasing amounts of GST-Syk, which contains the HRV 3C cleavage site. After a 16-hour incubation at 4°C, 1µg of GST-Syk was analyzed by SDS-PAGE. Two distinct cleavage products were seen.

## Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 88946 | **Pierce HRV 3C Protease**<br>*Sufficient for: Cleavage of 100mg of target protein at 1:200 enzyme-to-substrate ratio*<br>Kit Contents: HRV 3C Protease (2U/µL), 500µL<br>Reaction Buffer (10X), 10mL<br>GST-Syk Fusion Protein (1µg/µL), 25µL | 1,000 Unit Kit |
| 88947 | **Pierce HRV 3C Protease**<br>*Sufficient for: Cleavage of 1,000mg of target protein at 1:200 enzyme-to-substrate ratio*<br>Kit Contents: HRV 3C Protease (2U/µL), 5mL<br>Reaction Buffer (10X), 10mL<br>GST-Syk Fusion Protein (1µg/µL), 25µL | 10,000 Unit Kit |

# Accessory products


## Cell Lysis Reagents

Whole cell lysis buffers for high-yield protein extraction and solubilization from many cell and tissue types.

### Ordering Information

| Product # | Description | Pkg. Size |
|---|---|---|
| 89901 | RIPA Lysis and Extraction Buffer | 250mL |
| 78501 | M-PER™ Mammalian Protein Extraction Reagent | 250mL |
| 78505 | M-PER Mammalian Protein Extraction Reagent | 1000mL |
| 78510 | T-PER™ Tissue Protein Extraction Reagent | 500mL |
| 78835 | NE-PER™ Nuclear and Cytoplasmic Extraction Reagent Kit | 75mL kit |
| 90084 | B-PER Bacterial Protein Extraction Reagent | 250mL |
| 78248 | B-PER Bacterial Protein Extraction Reagent | 500mL |
| 78266 | B-PER Reagent (in Phosphate Buffer) | 500mL |
| 78260 | B-PER II Bacterial Protein Extraction Reagent (2X) | 250mL |
| 90079 | B-PER with Enzymes Bacterial Protein Extraction Kit | 500mL kit |
| 90081 | B-PER Direct Bacterial Protein Extraction Kit | 250mL kit |
| 88702 | Pierce Universal Nuclease for Cell Lysis, 99% purity | 100kU |
| 89802 | I-PER™ Insect Protein Extraction Reagents | 250mL |
| 78990 | Y-PER™ Yeast Protein Extraction Reagent | 500mL |
| 78999 | Y-PER Plus Dialyzable Yeast Protein Extraction Reagent | 500mL |

## Endotoxin Removal Resins

Reduces endotoxin levels in protein samples by 99% in 1 hour.

| Product # | Description | Pkg. Size |
|---|---|---|
| 88270 | High Capacity Endotoxin Removal Resin | 10mL |
| 88271 | High Capacity Endotoxin Removal Resin | 100mL |
| 88272 | High Capacity Endotoxin Removal Resin | 250mL |
| 88273 | High Capacity Endotoxin Removal Spin-Columns, 0.25mL | 5 columns |
| 88274 | High Capacity Endotoxin Removal Spin-Columns, 0.5mL | 5 columns |
| 88275 | High Capacity Endotoxin Removal Spin-Columns, 0.5mL | 25 columns |
| 88276 | High Capacity Endotoxin Removal Spin-Columns, 1mL | 5 columns |
| 88277 | High Capacity Endotoxin Removal Spin-Columns, 1mL | 25 columns |
| 88282 | LAL Chromogenic Endotoxin Quantitation Kit | 50 rxn. kit |

## Protease and Phosphatase Inhibitors

Ready-to-use inhibitor cocktails protect proteins from proteolysis and dephosphorylation.

| Product # | Description | Pkg. Size |
|---|---|---|
| 87786 | Halt Protease Inhibitor Cocktail (100X) | 1mL |
| 78429 | Halt Protease Inhibitor Cocktail (100X) | 5mL |
| 78438 | Halt Protease Inhibitor Cocktail (100X) | 10mL |
| 87785 | Halt Protease Inhibitor Cocktail, EDTA-Free (100X) | 1mL |
| 78437 | Halt Protease Inhibitor Cocktail, EDTA-free (100X) | 5mL |
| 78439 | Halt Protease Inhibitor Cocktail, EDTA-free (100X) | 10mL |
| 78444 | Halt Protease and Phosphatase Inhibitor Cocktail (100X) | 5 x 1mL |
| 78445 | Halt Protease and Phosphatase Inhibitor Cocktail, EDTA-free (100X) | 5 x 1mL |
| 78446 | Halt Protease and Phosphatase Inhibitor Cocktail (100X) | 10mL |
| 78447 | Halt Protease and Phosphatase Inhibitor Cocktail, EDTA-free (100X) | 10mL |
| 88265 | Pierce Protease Inhibitor Tablets | 20 tablets |
| 88266 | Pierce Protease Inhibitor Tablets, EDTA-free | 20 tablets |

## Protein Quantitation Assays

Measure total protein concentration after cell lysis, labeling or purification.

| Product # | Description | Pkg. Size |
|---|---|---|
| 22662 | Pierce 660nm Protein Assay Kit | 450mL kit |
| 23225 | BCA Protein Assay Kit | 1L kit |
| 23236 | Coomassie Plus (Bradford) Assay Kit | 950mL kit |

## Fusion and Epitope Tag Antibodies

Select the epitope and fusion tag antibodies you want for the easy detection of tagged proteins.

| Product # | Description | Pkg. Size |
|---|---|---|
| MA1-91878 | Anti-DYKDDDDK Tag (FLAG™ Epitope) | 100µg |
| MA1-21315 | Anti-His Epitope Tag (6xHis or HHHHHH) | 100µg |
| 26183 | Anti-HA Epitope Tag | 100µg |
| MA1-21316 | Anti-Myc Epitope Tag | 100µg |
| MA5-15253 | Anti-V5 Epitope Tag (GKPIPNPLLGLDST) | 100µg |
| MA4-004 | Anti-GST Tag (Glutathione S-Transferase) | 100µg |
| MA5-15256 | Anti-GFP Tag (Green Fluorescent Protein) | 100µg |
| MA5-15257 | Anti-RFP Tag (Red Fluorescent Protein) | 100µg |
| PA1-989 | Anti-MBP Tag (Maltose Binding Protein) | 100µg |
| CAB1001 | Anti-TAP Tag (Tandem Affinity Purification) | 50µg |


Protein Purification Technical Handbook


Western Blotting Handbook and Troubleshooting Tools

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