

molecular biology

Superior Performance of HIS-Select™ HF Nickel Affinity Gel

By John Dapron, Michael Harvey, Heidi Heimlich, Jon Stephan, Rick Mehig, and Bill Kappel
Sigma-Aldrich Corporation, St. Louis, MO, USA

Application Notes

- Highly cross-linked agarose suitable for FPLC, allowing higher flow rates and mechanical stability under pressure
- Non-charged hydrophilic linkage reduces non-specific binding
- High binding capacity of ≥ 15 mg/ml using histidine-tagged protein

Introduction

The expression and purification of histidine-tagged fusion proteins has become commonplace in recombinant protein expression and proteomic applications. Purification is routinely accomplished by immobilized metal affinity chromatography (IMAC), using nickel as the affinity ligand. Sigma-Aldrich has been expanding its line of specialty HIS-Select nickel chelate affinity media to meet the varied needs of the research community. All HIS-Select media incorporate our exclusive (patent pending) nitriloacetic acid (NTA) analog, which incorporates a tetradentate-chelated nickel with neutral spacer linkage. These attributes ensure high selectivity for histidine-tagged proteins, while maintaining good capacity and stability. One of the recent additions to this growing product line is HIS-Select Highflow (HF) Nickel Affinity Gel (Product Code [H 0537](#)), which complements our standard resin by providing greater structural stability. This resin is based on a highly cross-linked beaded agarose that is well suited for larger scale processing or small-scale fast performance liquid chromatography (FPLC) applications at pressures up to 200 psi. As with all HIS-Select resins, the HF matrix demonstrates superior performance for selectivity, particularly evident at lower expression levels or sub-saturating conditions of target protein. This article will illustrate the superior performance attributes of this specialty resin.

Superior selectivity

Commonly, nickel chelate resins based on iminodiacetic acid (IDA), or other versions of NTA type chelates, require supplements to reduce intrinsic non-specific binding. The typical supplement is imidazole, with recommended additions from 10-40 mM during load and wash conditions. The following experimental data demonstrates the excellent selectivity of the HIS-Select HF Nickel Affinity Gel compared to other commercial offerings. Highly cross-linked agarose based resins (HF type) typically demonstrate slightly higher non-specific interactions than low cross-linked agarose resins, but the increased structural rigidity allows utilization in specialized applications requiring medium pressure.

In order to test selectivity of different resins, purified histidine-tagged target protein (27 kDa) was spiked into an *E. coli* cell lysate and loaded at concentrations of 2 mg and 20 mg target protein per ml resin. Resins tested include two highly cross-linked media: HIS-Select HF Nickel Affinity Gel and Competitor HF NTA, plus three standard (low cross-linked) media: HIS-Select™ HC Nickel Affinity Gel (Product Code [P 6611](#)), Competitor NTA, and Competitor IDA. The resins were loaded and washed with 50 mM NaPO_4 , 300 mM NaCl, pH 8.0 (column buffer) containing 0 or 10 mM imidazole. Bound protein was eluted with column buffer containing 250 mM imidazole and analyzed by SDS-PAGE (Figure 1).

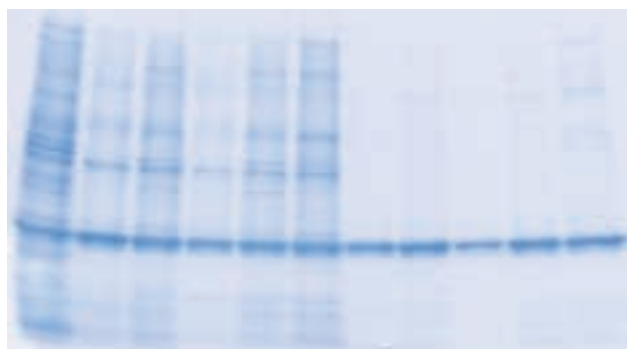
The stained protein gels indicate that when target protein is present at sub-saturating levels (2 mg target protein per ml resin), HIS-Select HF Nickel Affinity Gel and HIS-Select HC Nickel Affinity Gel display greater selectivity for the target protein compared to their respective competitors. This is especially evident in the absence of imidazole. Even when target protein is present at saturating levels (20 mg target protein per ml resin), the HIS-Select products are more selective for the target protein than the competitor products. This data demonstrates that the HIS-Select products allow greater specificity than the competitors. In situations when low expression results in sub-saturating load conditions, the benefits of low imidazole are more pronounced with HIS-Select HF Nickel Affinity Gel.

High mechanical strength

High-throughput technology for the purification of protein is defined by speed. When purifying multiple protein samples or a large volume of any one protein sample, increased throughput is the goal. FPLC applications often involve higher flow rates as a means to increase throughput. However, increases in flow rates result in pressure increases, and this requires a certain level of mechanical stability within the resin. The HIS-Select HF Nickel Affinity

2 mg/ml Target Protein Load

Load	0 mM imidazole					10 mM imidazole				
	Sigma	Comp	Sigma	Comp	Comp	Sigma	Comp	Sigma	Comp	Comp
	H 0537	HF	P 6611	NTA	IDA	H 0537	HF	P 6611	NTA	IDA



20 mg/ml Target Protein Load

Load	0 mM imidazole					10 mM imidazole				
	Sigma	Comp	Sigma	Comp	Comp	Sigma	Comp	Sigma	Comp	Comp
	H 0537	HF	P 6611	NTA	IDA	H 0537	HF	P 6611	NTA	IDA

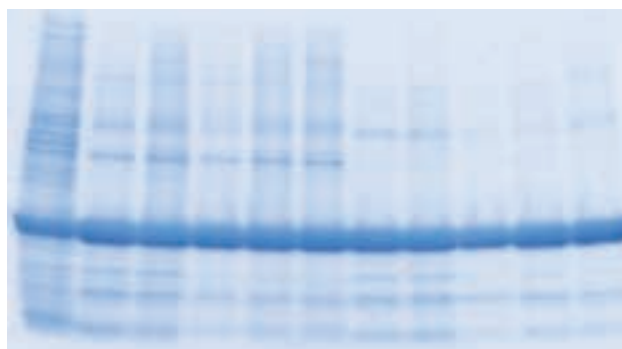


Figure 1. Selectivity comparison. Lysates from *E. coli* containing histidine-tagged protein were purified using HIS-Select HF Nickel Affinity Gel (Product Code [H 0537](#)), Competitor (Comp) HF, HIS-Select HC Nickel Affinity Gel (Product Code [P 6611](#)), Comp NTA, and Comp IDA. Lysed cells containing either 2 mg or 20 mg target protein per ml resin were loaded and washed with 50 mM sodium phosphate, 300 mM sodium chloride, pH 8, containing either 0 or 10 mM imidazole. HIS-tagged proteins were eluted with 50 mM sodium phosphate, 300 mM sodium chloride, pH 8, containing 250 mM imidazole. Equivalent volumes of each eluted sample were analyzed by SDS-PAGE and proteins visualized by EZBlue™ Gel Staining Reagent (Product Code [G 1041](#)).

Gel, created from highly cross-linked agarose, provides this mechanical stability and is suitable for use in FPLC applications. These applications are routinely run at a maximum linear flow rate of 3,000 cm/hr with average pressures as high as 150 psi. Large-scale applications can also benefit from medium pressure capabilities. A large-scale column can generate significant back pressure during operation. The HIS-Select HF is formulated for such applications. This resin will perform optimally even at a maximum linear flow rate of 3,000 cm/hr and with minimal compression at pressures up to 200 psi.

The mechanical stability of the HIS-Select HF Nickel Affinity Gel was evaluated by monitoring the compressibility of the resin at various pressures. The results of this evaluation are shown in Figure 2. The HIS-Select HF resin was compared to a Competitor HF resin along with traditional, low cross-linked HIS-Select and Competitor NTA resins. The percent compressibility of each of these resins was measured at pressures of 50, 100, 140, and 200 psi. Figure 2 indicates that both types of HF resins have much greater mechanical stability at the various pressures than the traditional, low cross-linked agarose based resins. HIS-Select HF and Competitor HF resins demonstrated less than 1% resin compression at pressures up to 140 psi, whereas the traditional resins demonstrated over 4% compression. Even at 200 psi the HF resins showed less than 2% compression compared to greater than 5% for the traditional resins. Importantly, the linear flow rate of the HF resins was approximately 980 cm/hr at 140 psi compared to an average 575 cm/hr for the traditional resins at the same pressure. Therefore, the HIS-Select HF resin may be utilized at high flow rates under medium pressure and is well suited for the high-throughput purification of histidine-tagged proteins.

Superior affinity matrix

The HIS-Select HF Nickel Affinity Gel was introduced to complement the existing agarose-based affinity resins. The HF resin shares the unique nickel chelate chemistry of the HIS-Select line of products, thus assuring optimum selectivity, while providing increased structural rigidity suitable for medium pressure applications. It is a superior affinity matrix for large scale and/or rapid (high-throughput) purification of recombinant proteins with histidine-containing tags.

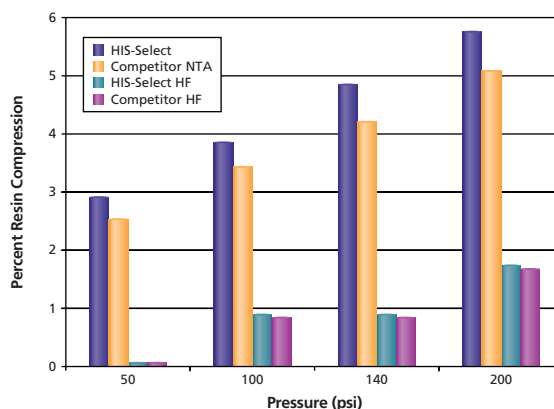


Figure 2. Compression comparison. Each resin type was evaluated by packing 1.0 ml of resin into a 1.2 ml FPLC Column (5 mm x 75 mm). The initial flow rate was adjusted to give a net pressure of 50 psi. Pressure and compression were then monitored every 5 minutes until stabilized at 100, 140, and 200 psi. Columns were run using 50 mM Sodium Phosphate, 250 mM Imidazole, 300 mM NaCl, pH 8.0.

Ordering Information

Product	Description	Unit
H 0537	HIS-Select™ HF Nickel Affinity Gel	10 ml 25 ml 100 ml 500 ml