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Introduction

Genaxxon His Affinity Agarose

The polyhistidine tag is the most widely used affinity tag due to its small size, low immunogenicity, and versatility under native and denaturing conditions, as well as in presence of detergents and many other additives. Taking advantage of the affinity of transition metal ions for the imidazole ring of histidine, immobilized metal affinity chromatography (IMAC) is used to purify his-tagged proteins. Genaxxon offers high-performance IDA Agarose and NTA Agarose, both based on BioWorks Workbeads.

Physically and chemically stable agarose matrix

Agarose bead size homogeneity affords high reproducibility

Both IDA and NTA chelating ligands available

Can be loaded with a metal ion of choice or ordered unloaded

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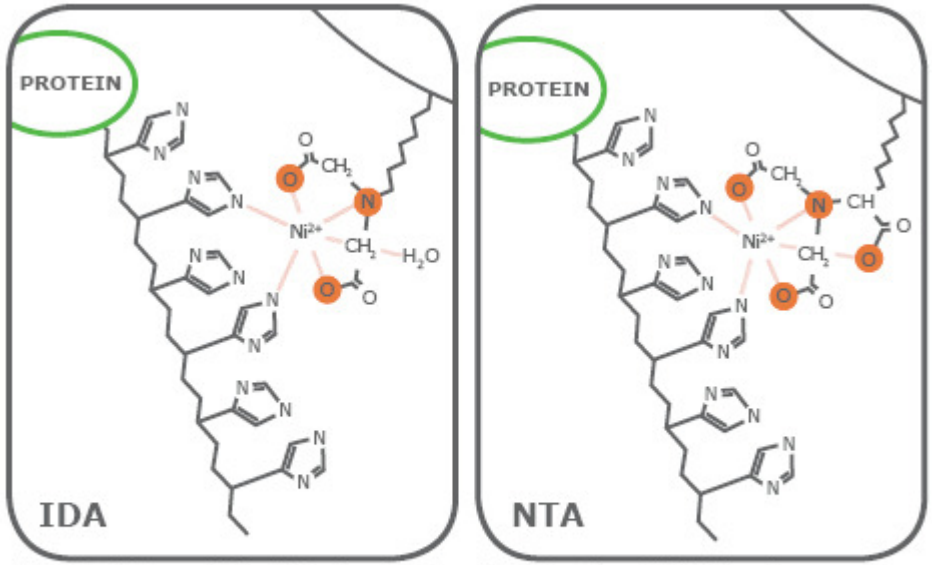

Fig. 1. NTA vs. IDA. Chelating ligands nitrilotriacetic acid (NTA) and iminodiacetic acid (IDA) support similar interaction between Ni^{2+} and imidazole rings of a polyhistidine tag, but NTA coordinates the Ni^{2+} with 4 valencies and IDA with only 3 (orange circles). This difference impacts the quality of the resulting purified protein fraction.

IDA versus NTA

As depicted in Figure 3, the chelating ligands iminodiacetic acid (IDA) and nitrilotriacetic acid (NTA) support similar interaction between nickel and the imidazole rings of the histidine tag. The ligands differ in size on the one hand; the smaller IDA couples to the resin at a higher density and therefore has higher binding capacity. On the other hand, IDA coordinates the nickel ion with one less valency than NTA (orange circles). As a result, more metal ions may leach from IDA resins giving a less pure eluted protein

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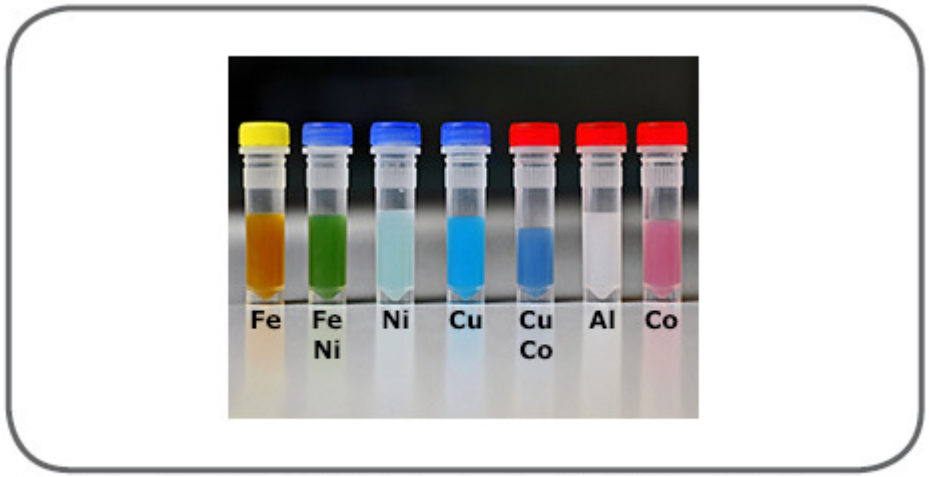

Fig. 2: Genaxxon His Affinit Resins feature flexible ligand loading. Both IDA and NTA resins can be loaded with the metal ion most suitable for a given application (Fe: iron, Ni: nickel, Cu: copper, CO: cobalt, Al: aluminium).

Flexible metal loading

Genaxxon IDA and NTA resins are standardly loaded with nickel for purification of his-tagged proteins. However, for applications requiring different protein specificity, such as for phosphorylated or zinc-finger proteins, or for modified selectivity towards his-tagged proteins, both resins can be loaded with other metal ions upon request. Figure 4 shows resins loaded with nickel, iron, copper, cobalt, aluminium, and combinations thereof. Alternatively, unloaded Genaxxon IDA or NTA Agarose is also available.

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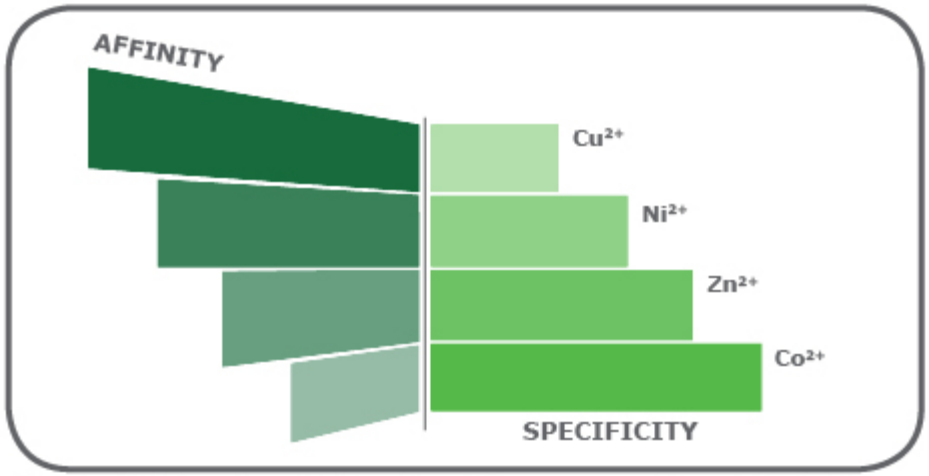

Fig. 3: Affinity and specificity of metal ions commonly used for IMAC. Loading an IDA or NTA resin with different metal ions can adjust the affinity and specificity of the resin to optimize the purity and yield of a purified protein.

Different metal ions confer different binding affinity and specificity

Loading different metal ions to a resin results in differing affinity and specificity for a his-tagged protein. Generally, cobalt exhibits the highest binding specificity of commonly used IMAC metal ions, leading to relatively low yields but high purity. Copper, at the other end of the spectrum, has a high affinity leading to high yields but unspecific binding. Different metals are optimal for different proteins. For example, zink is commonly used for zink-finger proteins, trivalent ions such as aluminium and iron are used for phosphorylated proteins. In searching for the optimal resin to purify a protein, it is recommended to explore different chelating ligands (IDA or NTA) and different metal ions.

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Comparison between Ni-IDA and Ni-NTA from Genaxxon and competitors

Genaxxon Ni-IDA and Ni-NTA Agarose

The His tag is the most widely used affinity tag due to its small size, low immunogenicity, and versatility under native or denaturing conditions, as well as in presence of detergents and many other additives. Genaxxon offers high-performance IDA- and NTA Agarose, based on BioWorks Workbeads, for purification of his-tagged proteins.

- Ni-NTA with the highest binding capacity on the market (up to 80mg/mL)
- Ni-IDA with a binding capacity of up to 100mg/mL
- Ni-NTA shows superior DTT and EDTA stability
- Ni-IDA is stable und commonly used concentrations of DTT and EDTA
- Can be regenerated for reuse

Note:
Genaxxon NTA and IDA Agarose is provided as a 50% suspension.

Genaxxon NTA and IDA Agarose (loaded with your metal ion of choice) is also available as prepacked chromatography columns.

For purification of his-tagged proteins from cell culture supernatants or for pull-down experiments, we recommend Genaxxon His Affinity MagBeads.

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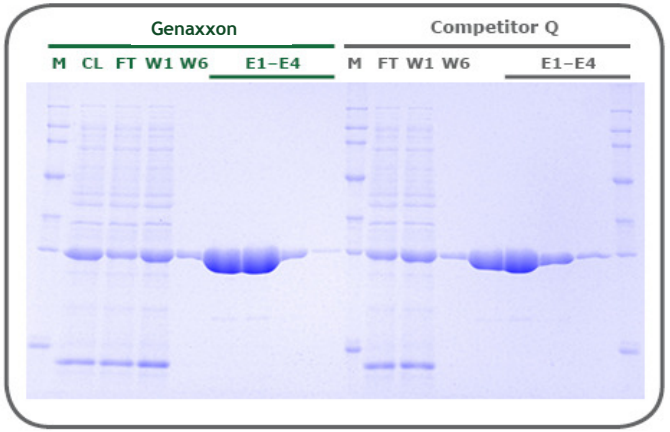

Fig. 1: Over 20% more yield obtained with Genaxxon Ni-NTA Agarose.
SDS-PAGE of GFP expressed in *E.coli* and purified in gravity columns with Genaxxon Ni-NTA Agarose and Ni-NTA resin from Competitor Q. 80 mg/mL protein yield was obtained with Genaxxon Ni-NTA Agarose (E1-E4, Genaxxon) compared to 65 mg/mL with the widely used alternative resin (E1-E4, Competitor Q).

Highest binding capacity on the market

Our unique production process yields a Ni-NTA Agarose that exhibits a protein binding capacity >20% higher than that of two leading competitor products. Figure 1 shows the SDS-PAGE of GFP expressed in E. coli and purified in gravity colums with Genaxxon Ni-NTA Agarose and the Ni-NTA resin from Competitor Q. The protein yield in 4 elutions (E1-E4) was 80mg/mL, compared to 65mg/mL obtained with the alternative resin (E1-E4, Competitor Q). Similar results (10% higher binding capacity; data not shown here) were obtained comparing the purification of JNK1 (Kinase, 48 kDa) on PureCube Ni-NTA and the Ni-NTA of another leading provider. Table 1 compares additional parameters among Genaxxon Ni-NTA Agarose and two equivalent market-leading resins.

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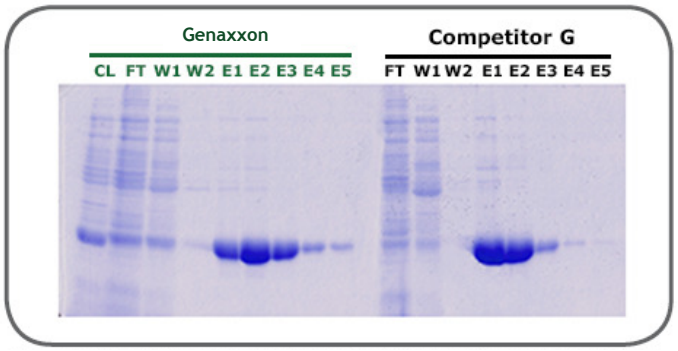

Fig. 1: The binding capacity of Genaxxon Ni-IDA Agarose is comparable to resins from a leading supplier. SDS-PAGE of CAT purified in gravity columns with Genaxxon Ni-IDA Agarose and Ni-Sepharose® resin from Competitor G show elution fractions (E1-E5) with similar protein yields.

Strong and robust performance

Based on the same agarose matrix with high porosity and physical stability, Genaxxon Ni-IDA agarose exhibits a protein capacity of up to 100mg protein per mL resin, which is competitive with that of products from market-leading providers. Figure 2 shows the SDS-PAGE analysis of 6-His chloramphenicoltransferase (CAT) expressed in E. coli and purified on gravity flow columns filled with Genaxxon Ni-IDA Agarose or Competitor G Ni-Sepharose High Performance resin. The amount of protein drawn down from the columns in 5 elution fractions was comparable for the two affinity resins (CL: cleared lysate; FT: flow-through; W1-2: wash fractions; E1-5: elution fractions). 2 compares additional parameters among Genaxxon Ni-NTA Agarose and two equivalent market-leading resins.

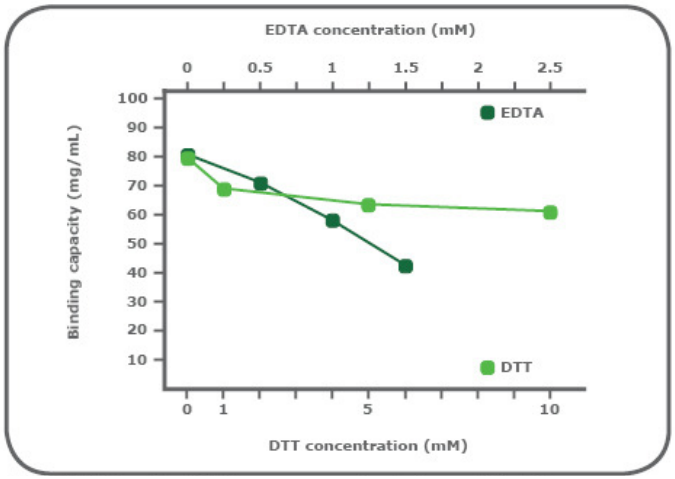

Fig. 2: NTA is robust in the presence of reducing and chelating agents. GFP-His was purified on gravity columns containing Genaxxon Ni-NTA Agarose after exposing the resin for 1 h to 3 concentrations of DTT or EDTA. NTA exhibits a shallow decay rate in binding capacity.

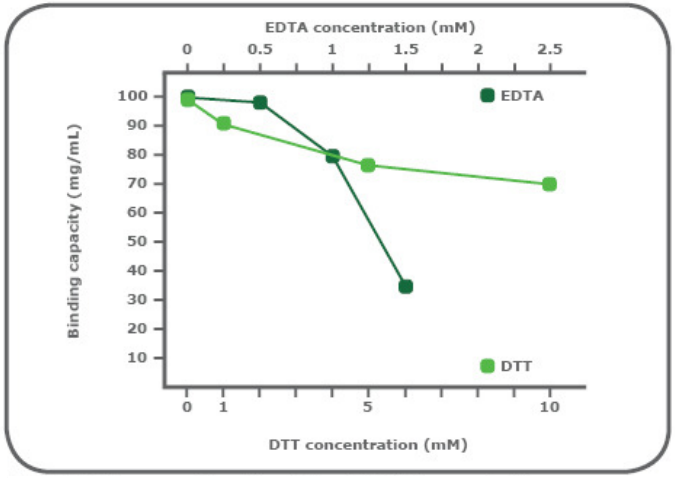

Fig. 2: Binding capacity of Genaxxon Ni-IDA Agarose in the presence of reducing and chelating agents. GFP-His was purified on gravity columns containing Genaxxon Ni-IDA Agarose after exposing the resin for 1 h to 3 concentrations of DTT or EDTA. Binding capacity remains relatively stable with DTT and decays first when EDTA concentration is >1.0 mM.

Superior DTT and EDTA stability

Genaxxon Ni-NTA Agarose is very robust in the presence of DTT and EDTA. In a stability test, Genaxxon Ni-NTA Agarose was exposed to increasing concentrations of DTT or EDTA for 1h. Thereafter, the resins were used to purify E. coli-expressed GFP-His in gravity columns. The binding capacity of the resin decreased in the presence of both DTT and EDTA but the decay rate was shallow. In presence of DTT, Genaxxon Ni-NTA Agarose lost on average 8% binding capacity with each increase in DTT concentration, resulting in an overall decay of 22% at 10 mM. The loss in binding capacity was steeper with EDTA, however the resin still exihibits 54% of its maximum binding capacity at an EDTA concentration of 1.5mM. Table 1 summarizes stability values for Genaxxon Ni-NTA Agarose and 2 equivalent market-leading resins.

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Compatible with reagents and additives

The impact of the reducing agent DTT and metal chelator EDTA on the binding capacity of Genaxxon Ni-IDA Agarose has been examined. After exposing the resin for 1h to different concentrations of the two agents, GFP-His was purified on the treated resins. Binding capacity declines with increasing concentration of each reagent but the decay rate is relatively shallow in the presence of DTT (overall decline of 30%) and up to 1mM EDTA (20% decline). Table 2 summarizes stability values for Genaxxon Ni-IDA Agarose and 2 equivalent market-leading resins.

	Genaxxon	Competitor G	Competitor Q
Particle size	32-60 µm	average 90 µm	60-160 µm
Metal ion capacity (Cu, Ni)	>15 µmol/mL	~15 µmol/mL	No information
Binding capacity	up to 80 mg/mL	>40 mg/mL	<50 mg/mL
pH stability (processing)	3.0-12.0	3.0-12.0	No information
Recommended flow rate	0.5-2.0 mL/min (6.0 mL/min poss.)	1.0 mL/min	1.0-3.0 mL/min
DTT stability	<10 mM	<5 mM	<10 mM
EDTA stability	<1.5 mM	<1 mM	<1 mM

Table 1. Technical and performance parameters of 3 Ni-NTA agarose resins.

	Genaxxon	Competitor G	Competitor Q
Particle size	32-60 µm	average 90 µm	45-160 µm
Metal ion capacity (Cu, Ni)	>25 µmol/mL	>30 µmol/mL	6-18 µmol/mL
Binding capacity	up to 100 mg/mL	No information	< 15 mg/mL
pH stability (processing)	3.0-12.0	3.0-13.0	No information
Recommended flow rate	0.5-2.0 mL/min (6.0 mL/min poss.)	1.0 mL/min	1.0 mL/min
DTT stability	<10 mM	No information	5 mM
EDTA stability	<1.0 mM	No information	Not recommend

Table 1. Technical and performance parameters of 3 Ni-NTA agarose resins.

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Top material quality, unrivaled performance

Genaxxon Ni-NTA Agarose is produced with a highly cross-linked agarose which is physically very stable and suitable for purification processes under low pressure (bulk purifications as well as packed in chromatography columns). Additionally, the high porosity and uniform size of the matrix allows for optimal protein interaction and high reproducibility between individual purification runs. Table 1 summarizes technical and performance parameters of Genaxxon Ni-NTA Agarose and 2 equivalent, market-leading resins.

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Top-quality materials, competitive performance

Genaxxon Ni-IDA Agarose is produced with a highly cross-linked agarose which is physically very stable and suitable for purification processes under low pressure (bulk purifications as well as packed in chromatography columns). Additionally, the high porosity and uniform size of the matrix allows for optimal protein interaction and high reproducibility between individual purification runs. Table 1 summarizes technical and performance parameters of Genaxxon Ni-IDA Agarose and 2 equivalent, market-leading resins

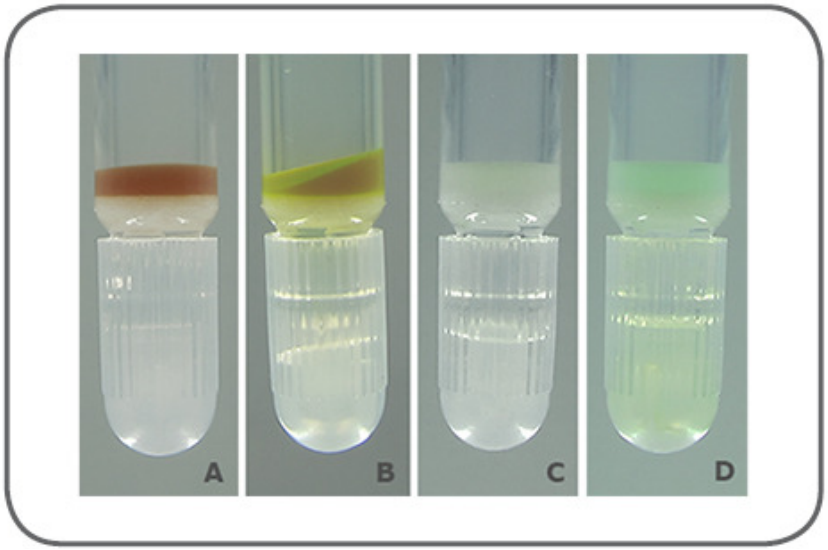

Fig. 3: Genaxxon Ni-IDA Agarose is easily regenerated. Genaxxon Ni-IDA Agarose was exposed to 5mM DTT for 8 h **(A)**. After demonstrating that it could still bind GFP **(B)**, the resin was washed, stripped **(C)**, and reloaded with Ni^{2+} **(D)** following standard Genaxxon protocol (see Genaxxon Protocols & Datasheets).

Regenerable resins for reuse

Strong reducing agents such as DTT will reduce nickel ions and turn resins brown. Genaxxon Ni-IDA Agarose can tolerate exposure to 5 mM DTT and still bind protein (**B**). Furthermore, the resins can be regenerated. In figure 3, Genaxxon Ni-IDA Agarose exposed to 5mM DTT for 8 hours (**A**) could still visibly bind green fluourescent protein (**B**). To regenerate the resin, the chelating ligand (IDA or NTA) is stripped of nickel, turning the resin white (**C**), and is then reloaded with nickel ions whereby the resin reacquires its characteristic blue-green color (**D**).

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