

# Ni-IDA/ Ni-NTA MagBeads His-Tag

## Purification of His-tagged Proteins

Product	Cat#	Package size
Ni-IDA MagBeads His-Tag 25% suspension	S5388.0001	1mL
Ni-IDA MagBeads His-Tag 25% suspension	S5388.0005	5mL
Ni-IDA MagBeads His-Tag 25% suspension	S5388.0025	25mL
Ni-NTA MagBeads His-Tag 25% suspension	S5390.0001	1mL
Ni-NTA MagBeads His-Tag 25% suspension	S5390.0005	5mL
Ni-NTA MagBeads His-Tag 25% suspension	S5390.0025	25mL

		page
1.	Overview	1
1.1	Specifications	2
2.	Purification of His-tagged Proteins under native conditions using Genaxxon Ni-IDA or Ni-NTA MagBeads	2
2.1	Equipment and Materials	2
2.2	Solutions and Buffers	2
2.3	Procedure	3
3.	Purification of His-tagged Proteins under denaturing conditions using Genaxxon Ni-IDA or Ni-NTA MagBeads	4
3.1	Equipment and Materials	5
3.2	Solutions and Buffers	5
3.3	Procedure	6
4.	Washing and Regenerating Ni-NTA and Ni-IDA MagBeads	7
4.1	Equipment and Materials	7
4.2	Solutions and Buffers	7
4.3	Procedure	8
5.	Technical and Performance Parameters	9
6.	References	9
7.	Important Information	9
8.	Warranty	9
9.	Safety information	9

### 1. Overview

Magnetic beads are ideal for protein purification from dilute supernatants and for pull-down experiments. Genaxxon MagBeads are ferrimagnetic agarose beads coupled to a chelating ligand (IDA or NTA) coordinating nickel ions. Both ligand-metal systems efficiently bind histidine-tagged proteins.

Protein purification based on magnetic beads has become popular because they are useful to

- extract proteins from diluted solutions, such as cell culture supernatants
- highly reproducible results with low unspecific binding supernatants
- homogeneous in size and low lot-to-lot variation
- purify proteins expressed at low levels
- suited for purification from dilute samples and pull-down experiments

Genaxxon MagBeads enable fast and easy purification steps, which can be automated. The amount of magnetic beads used for a purification setup can be easily scaled up and down to match protein expression rates and culture volumes.

## 1.1 Specifications

particle size	30µm
pH stability	2.0-4.0
formulation	unbuffered suspension in 20% ethanol
binding capacity* Genaxxon Ni-IDA MagBeads	40mg/mL settled beads
binding capacity* Genaxxon Ni-NTA MagBeads	70mg/mL settled beads
antimicrobial agent	20% ethanol
stability	2 years
storage	2 °C - 8 °C, do not freeze!

\*as determined by purification of 6xHis-tagged GFP protein from *E. coli* cleared lysates, and quantified via spectrophotometry.

## 2. Purification of His-tagged Proteins under native conditions using Genaxxon Ni-IDA or Ni-NTA MagBeads

This protocol describes the generation of a cleared lysate from an *E. coli* cell pellet and the subsequent purification of His-tagged proteins under native conditions using Genaxxon Ni-IDA or Ni-NTA MagBeads. Reagent amounts given apply to 10mL IPTG-induced bacterial culture of a well-expressed protein (approximately 10-50mg/L). Magnetic bead purification is easily scalable. To minimize unspecific binding and reduce cost, the volume magnetic bead suspension used should be adjusted to the expression level of interest. See table 1 for more details.

In this protocol, cell lysis is done using lysozyme because it is an inexpensive and efficient method for cells that have been frozen. However, lysis methods using detergents (e.g. CHAPS) can also be used. The His-tagged target protein is purified from cleared lysate under native conditions in a bind-wash-elute procedure. Magnetic beads are well-suited to purify proteins from dilute solutions, such as cell culture or medium supernatants. Please contact us if you have questions or need assistance optimizing a protocol for your application ([info@genaxxon.com](mailto:info@genaxxon.com)).

### 2.1

Equipment	Materials
<input type="checkbox"/> Ice bath <input type="checkbox"/> Refrigerated centrifuge (min 10,000xg) <input type="checkbox"/> Micropipettor <input type="checkbox"/> Micropipetting tips <input type="checkbox"/> 1.5mL conical microcentrifuge tubes <input type="checkbox"/> Magnetic holder for microcentrifuge tubes (for separation of magnetic beads) <input type="checkbox"/> pH meter <input type="checkbox"/> End-over-end shaker	<input type="checkbox"/> Cell pellet from expression screen (e.g., from 10mL culture) <input type="checkbox"/> Sodium phosphate monobasic (NaH <sub>2</sub> PO <sub>4</sub> ) <input type="checkbox"/> Sodium chloride (NaCl) <input type="checkbox"/> Imidazole <input type="checkbox"/> Sodium hydroxide (NaOH) <input type="checkbox"/> Lysozyme <input type="checkbox"/> Benzonase® nuclease (e.g., Novagen®) <input type="checkbox"/> Genaxxon Ni-IDA MagBeads (1mL; Genaxxon #S5388) or Genaxxon Ni-NTA MagBeads (1mL; Genaxxon #S5390) <input type="checkbox"/> Dithiothreitol (DTT) <input type="checkbox"/> Glycerol <input type="checkbox"/> Sodium dodecyl sulfate (SDS) <input type="checkbox"/> Bromophenol blue <input type="checkbox"/> Tris base <input type="checkbox"/> HCl <input type="checkbox"/> Optional: Protease inhibitor cocktail without EDTA

needed but not supplied

## 2.2 Solutions and buffers

### Lysis Buffer, 10mL

component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for stock	Stock needed for buffer
NaH <sub>2</sub> PO <sub>4</sub>	50mM	119.98	0.5M	29.99g/500mL	1mL
NaCl	300mM	58.44	5M	146.1g/500mL	0.6mL
Imidazole	10mM	68.08	1M	6.8g/100mL	0.1mL
<b>Instructions:</b> Mix in 6mL water. Adjust the pH to 8.0 using NaOH and then add water to a total volume of 10mL. Always prepare fresh.					

### Wash Buffer, 10 mL

component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for stock	Stock needed for buffer
NaH <sub>2</sub> PO <sub>4</sub>	50mM	119.98	0.5M	29.99g/500mL	1mL
NaCl	300mM	58.44	5M	146.1g/500mL	0.6mL
Imidazole	20mM	68.08	1M	6.8g/100mL	0.2mL
<b>Instructions:</b> Mix in 6mL water. Adjust the pH to 8.0 using NaOH and then add water to a total volume of 10mL. Always prepare fresh.					

### Elution Buffer, 10mL

component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for stock	Stock needed for buffer
NaH <sub>2</sub> PO <sub>4</sub>	50mM	119.98	0.5M	29.99g/500mL	1mL
NaCl	300mM	58.44	5M	146.1g/500mL	0.6mL
Imidazole*	500mM	68.08	1M	6.8g/100mL	5mL
<b>Instructions:</b> Mix in 9.5mL water. Adjust the pH to 8.0 using NaOH and then add water to a total volume of 10mL. Always prepare fresh.					

\* Tag length and protein structure can impact the interaction between His-tag and nickel ion. Therefore, we recommend trying a concentration gradient of imidazole to find the minimum concentration that elutes the desired amount of protein from the column.

### 5X SDS-PAGE Buffer, 10mL

component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for stock	Stock needed for buffer
Tris-HCl, pH 6.8-7.0	300mM	121.14	1M	121.14g/1L	3mL
Glycerol	50% (v/v)	-	100% (v/v)	-	5mL
SDS	5% (w/v)	-	-	-	0.5g
Bromophenol blue	0.05% (w/v)	-	4%	-	125µL
DTT	250mM	154.25	1M	1.54g/10mL	125µL/aliquot
<b>Instructions:</b> Make sure to prepare a 1M Tris-HCl stock by dissolving Tris base in 500mL deionized water, adding HCl to a pH of 6.8-7.0, and adding water to a final volume of 1L. For the SDS-PAGE Buffer, mix all components listed <b>except</b> DTT and add water to a total of 10mL. Freeze 20 aliquots (0.5mL each) at -20°C. Before use, add DTT to the needed single aliquots.					

**Table 1: Magnetic bead suspension volumes suitable for given protein expression levels**

Protein expression level	Amount of His-tagged protein per 1mL culture	Amount of His-tagged protein per 10mL* culture	Volume 5% magnetic bead suspension per 10mL culture	Minimum elution volume per 10mL culture
<0.5mg/L	<0.5µg	<5µg	10µL	25µL
1mg/L	1µg	10µg	20µL	25µL
5mg/L	5µg	50µg	100µL	50µL
10mg/L	10µg	100µg	200µL	100µL
50mg/L	50µg	500µg	1mL	500µL

\* Volumes can be linearly scaled up or down for smaller or larger culture volumes.

## 2.3 Procedure

1. Thaw the E. coli cell pellet on ice.
2. Resuspend the cell pellet in 1mL Lysis Buffer supplemented with 1mg/mL lysozyme.
3. Add 6U Benzonase® (3 units/mL bacterial culture) to the lysate to reduce viscosity caused by genomic DNA.
4. Incubate for 30 min on ice, if necessary. Otherwise, incubating at room temperature (20-25°C) may be more efficient.
5. Centrifuge the lysate for 30 min at 10,000xg and 2-8°C. Collect the supernatant.

**Optional:** Add 1 tablet EDTA-free protease inhibitor cocktail to the Lysis Buffer.

**Optional:** Freezing the cell pellet at -20°C for 30 min prior to incubation at room temperature improves lysis by lysozyme.

**Note:** The supernatant contains the soluble proteins and is the cleared lysate.

6. Resuspend the Genaxxon Ni-IDA or Ni-NTA MagBeads by vortexing. Transfer 200µL of the 5% magnetic beads suspension into a conical microcentrifuge tube (or the volume adjusted to the expression level, see table 1).
7. Add 500mL Lysis Buffer and mix by vortexing. Place the tube on a magnetic microtube stand until the beads are separated and discard the supernatant.
8. Pipet 1mL of the cleared lysate onto the equilibrated magnetic beads, and incubate the lysate-magnetic bead mixture at 4°C for 1 h on an end-over-end shaker.
9. Place the tube on the magnetic microtube stand until the beads separate and remove the supernatant.
10. Remove the tube from the magnet. Add 500mL Wash Buffer and mix by vortexing. Place the tube again on the magnetic microtube stand and allow the beads to separate. Remove the supernatant.
11. Repeat step 10 twice.
12. Elute the His-tagged protein using 100µL Elution Buffer (or the volume adjusted to the expression level; see Table 1).
13. Repeat step 12. Collect each elution fraction in a separate tube and determine the protein concentration of each fraction.
14. We recommend saving small aliquots at various steps and of the collected fractions and analyzing them by SDS-PAGE and Western blot to assess the efficiency of the purification process.

**Tip:** Lysis Buffer contains 10mM imidazole to prevent binding of untagged proteins. If His-tagged proteins do not bind under these conditions, reduce the imidazole concentration to 1-5mM.

This is the **flow-through fraction**.

This is the first **wash fraction**.

This is the first **elution fraction**.

**Note:** Do not boil membrane proteins. Instead, incubate the sample at 46°C for 30 min in preparation for SDS-PAGE analysis.

### 3. Purification of His-tagged Proteins under denaturing conditions using Genaxxon Ni-IDA or Ni-NTA MagBeads

This protocol describes the generation of a cleared lysate from an *E. coli* cell pellet and the subsequent purification of His-tagged proteins under denaturing conditions using Genaxxon Ni-IDA or Ni-NTA MagBeads. Reagent amounts given apply to 10 mL IPTG-induced bacterial culture of a well-expressed protein (approximately 10-50mg/L). Magnetic bead purification is easily scalable. To minimize unspecific binding and reduce cost, the volume magnetic bead suspension used should be adjusted to the expression level of interest. See Table 2 for more details.

In this protocol cells are lysed with a high concentration of urea, which also aids to dissolve insoluble protein aggregates. The His-tagged protein is purified from the cleared lysate under denaturing conditions in a bindwash- elute procedure. Binding occurs at slightly alkaline pH, while washing and elution are done with a stepwise pH decrease.

Magnetic beads are well-suited to purify proteins from dilute solutions, such as cell culture or medium supernatants. Please contact us if you have questions or need assistance optimizing a protocol for your application ([info@genaxxon.com](mailto:info@genaxxon.com)).

## 3.1

Equipment	Materials
<input type="checkbox"/> Ice bath <input type="checkbox"/> Refrigerated centrifuge (min 10,000xg) <input type="checkbox"/> Micropipettor <input type="checkbox"/> Micropipetting tips <input type="checkbox"/> 1.5mL conical microcentrifuge tubes <input type="checkbox"/> Magnetic holder for microcentrifuge tubes (for separation of magnetic beads) <input type="checkbox"/> pH meter <input type="checkbox"/> End-over-end shaker	<input type="checkbox"/> Cell pellet from expression screen (e.g., from 10mL culture) <input type="checkbox"/> Sodium phosphate monobasic (NaH <sub>2</sub> PO <sub>4</sub> ) <input type="checkbox"/> Sodium chloride (NaCl) <input type="checkbox"/> Tris base <input type="checkbox"/> Urea <input type="checkbox"/> Hydrochloric acid (HCl) <input type="checkbox"/> Sodium hydroxide (NaOH) <input type="checkbox"/> Genaxxon Ni-IDA MagBeads (1mL of 5% solution; Genaxxon #S5388) or Genaxxon Ni-NTA <input type="checkbox"/> Agarose (1mL of 5% solution; Genaxxon #S5390) <input type="checkbox"/> Dithiothreitol (DTT) <input type="checkbox"/> Glycerol <input type="checkbox"/> Sodium dodecyl sulfate (SDS) <input type="checkbox"/> Bromophenol blue <input type="checkbox"/> Optional: Benzonase® nuclease (e.g., Novagen®)

needed but not supplied

## 3.2 Solutions and buffers

## Denaturing Lysis Buffer, pH8.0, 10mL

component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for stock	Stock needed for buffer
NaH <sub>2</sub> PO <sub>4</sub>	50mM	119.98	0.5M	29.99g/500mL	1mL
NaCl	300mM	58.44	5M	146.1g/500mL	0.6mL
Imidazole	10mM	68.08	1M	6.8g/100mL	0.1mL
Urea	8M	60.06	-	-	4.8g

**Instructions:** Dissolve in 5mL water and then bring volume to 9mL. Adjust pH to 8.0 with HCl and add water to a total volume of 10mL. Due to urea dissociation, adjust the pH immediately before use.

## Denaturing Wash Buffer, pH 6.3, 10mL

component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for stock	Stock needed for buffer
NaH <sub>2</sub> PO <sub>4</sub>	50mM	119.98	0.5M	29.99g/500mL	1.0mL
NaCl	300mM	58.44	5M	146.1g/500mL	0.6mL
Imidazole	20mM	68.08	1M	6.8g/100mL	0.2mL
Urea	8M	60.06	-	-	4.8g

**Instructions:** Dissolve in 5mL water and then bring volume to 9mL. Adjust pH to 6.3 with HCl and add water to a total volume of 10mL. Due to urea dissociation, adjust the pH immediately before use.

## Denaturing Elution Buffer, pH 4.5, 10mL

component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for stock	Stock needed for buffer
NaH <sub>2</sub> PO <sub>4</sub>	50mM	119.98	0.5M	29.99g/500mL	1.0mL
NaCl	300mM	58.44	5M	146.1g/500mL	0.6mL
Imidazole	250mM	68.08	1M	6.8g/100mL	2.5mL
Urea	8M	60.06	-	-	4.8g

**Instructions:** Dissolve in 5mL water and then bring volume to 9mL. Adjust pH to 4.5 with HCl and add water to a total volume of 10mL. Due to urea dissociation, adjust the pH immediately before use.

### 5X SDS-PAGE Buffer, 10mL

component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for stock	Stock needed for buffer
Tris-HCl, pH 6.8-7.0	300mM	121.14	1M	121.14g/1L	3mL
Glycerol	50% (v/v)	-	100% (v/v)	-	5mL
SDS	5% (w/v)	-	-	-	0.5g
Bromophenol blue	0.05% (w/v)	-	4%	-	125µL
DTT	250mM	154.25	1M	1.54g/10mL	125µL/aliquot

**Instructions:** Make sure to prepare a 1M Tris-HCl stock by dissolving Tris base in 500mL deionized water, adding HCl to a pH of 6.8-7.0, and adding water to a final volume of 1L. For the SDS-PAGE Buffer, mix all components listed **except** DTT and add water to a total of 10mL. Freeze 20 aliquots (0.5mL each) at -20°C. Before use, add DTT to the needed single aliquots.

**Table 2: Magnetic bead suspension volumes suitable for given protein expression levels**

Protein expression level	Amount of His-tagged protein per 1mL culture	Amount of His-tagged protein per 10mL* culture	Volume 5% magnetic bead suspension per 10mL culture	Minimum elution volume per 10mL culture
<0.5mg/L	<0.5µg	<5µg	10µL	25µL
1mg/L	1µg	10µg	20µL	25µL
5mg/L	5µg	50µg	100µL	50µL
10mg/L	10µg	100µg	200µL	100µL
50mg/L	50µg	500µg	1mL	500µL

\* Volumes can be linearly scaled up or down for smaller or larger culture volumes.

### 3.3 Procedure

1. Thaw the E. coli cell pellet on ice.
2. Resuspend the cell pellet in 1mL Denaturing Lysis Buffer.
3. Incubate at room temperature for 30 min on an end-over-end shaker.
4. Centrifuge the lysate for 30 min at room temperature and 10,000xg. Collect the supernatant.
5. Pipet 1mL of the cleared lysate into a conical microcentrifuge tube.
6. Resuspend the PureCube Ni-IDA or Ni-NTA MagBeads by vortexing. Transfer 200µL of the 5% magnetic beads suspension onto the lysate (or the volume adjusted to expression level; see Table 2).
7. Incubate the lysate-magnetic bead mixture at room temperature for 1 h on an end-over-end shaker.
8. Place the tube on the magnetic microtube stand until the beads separate and remove the supernatant.
9. Remove the tube from the magnet. Add 500µL Denaturing Wash Buffer and mix by vortexing. Place the tube again on the magnetic microtube stand and allow the beads to separate. Remove the supernatant.
10. Repeat step 9 twice.
11. Elute the His-tagged protein using 100µL Denaturing Elution Buffer (or the volume adjusted to the expression level; see Table 2).
12. Repeat step 11. Collect each elution fraction in a separate tube and determine the protein concentration of each fraction.
13. We recommend saving small aliquots at various steps and of the collected fractions and analyzing them by SDS-PAGE and Western blot to assess the efficiency of the purification process.

**Optional:** Benzonase® can be added to the lysate to reduce viscosity caused by nucleic acids (3 U/mL bacterial culture). Read “about denaturation”. In addition, nucleic acids can be sheared by passing the lysate 10 times through a fine-gauge needle.

**Note:** This crude lysate represents the **total cellular protein**.

**Note:** The supernatant contains the chaotropesoluble proteins and is the **cleared lysate**.

**Tip:** Briefly centrifuge the sample before placing it on the magnetic separator in order to collect liquid from the lid.

This is the **flow-through fraction**.

This is first the **wash fraction**.

This is the first **elution fraction**.

**Tip:** If the target protein is acid-labile, elution can be performed with 250-500 mM imidazole.

**Note:** Do not boil membrane proteins. Instead, incubate samples at 46°C for 30 min in preparation for SDS-PAGE analysis.

**About denaturation:**

In some cases 8 M urea is not sufficient to completely solubilize inclusion bodies. In these cases the urea in the Denaturing Lysis Buffer can be replaced with 6 M guanidine hydrochloride (Gu-HCl). **Important:** Samples containing Gu-HCl cannot be directly applied to SDS-PAGE. Dilute the sample or subject it to a precipitation step (e.g., using trichloroacetate (TCA) or similar) to remove the denaturant. If using Benzonase® to remove nucleic acids, the concentration of urea in the Denaturing Lysis Buffer must be decreased. Benzonase® is active only at urea concentrations  $\leq 7$  M. In contrast, Gu-HCl inactivates Benzonase® even at low concentrations.

#### 4. Washing and Regenerating Ni-NTA and Ni-IDA MagBeads

Ni-NTA and Ni-IDA MagBeads should be washed after each run and regenerated latest after 5 runs (though we recommend to regenerate the MagBeads after each run, if possible). This protocol delineates washing and regenerating procedures for Genaxxon Ni-NTA and Ni-IDA MagBeads, including a specific procedure for MagBeads that have been exposed to a reducing agent such as DTT. Volumes are given in *column bed volume* (bv), i.e., 10 bv calls for 10mL of buffer for a 1mL column bed volume. This protocol can also be implemented for NTA and IDA MagBeads loaded with other metals (e.g., Co, Fe, Al, Cu), using the appropriate solutions to recharge the MagBeads. Please contact us if you have questions or need assistance optimizing a protocol for your application ([info@genaxxon.com](mailto:info@genaxxon.com)).

##### 4.1

Equipment	Materials
<input type="checkbox"/> Disposable gravity flow columns (minimum 1mL bed volume)	<input type="checkbox"/> Sodium chloride (NaCl) <input type="checkbox"/> Sodium hydroxide (NaOH) <input type="checkbox"/> Ethylenediaminetetraacetic acid (EDTA) <input type="checkbox"/> N,N-Dimethyldodecylamine-N-oxide (LDAO, 1g) <input type="checkbox"/> 20% (v/v) Ethanol (C <sub>2</sub> H <sub>6</sub> O) <input type="checkbox"/> Nickel sulfate (NiSO <sub>4</sub> ) <input type="checkbox"/> Hydrochloric acid (HCl)

needed but not supplied

##### 4.2 Solutions and buffers

###### Wash Buffer, 10mL

component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for stock	Stock needed for buffer
NaOH	0.5M	39.997	1M	20.00g/500mL	5mL
NaCl	2.0M	58.440	10M	292.2g/500mL	2mL
LDAO*	2% (v/v)	229.40	30% (w/v)	0.3g/1mL	0.66mL

**Instructions:** Mix components. Add water to a final volume of 10mL. Note that LDAO is only required if a membrane protein was purified on the MagBeads being washed or regenerated.

\*LDAO is only required if a membrane protein was purified on the MagBeads being washed or regenerated. An alternate detergent may be used but generally we recommend LDAO because it is non-ionic, harsh to proteins, and easily washed off the MagBeads.

###### 100 mM EDTA, 100mL

component	Final concentration	Molecular weight (g/mol)	Amount needed
EDTA	100mM	292.24	2.922g/100mL

**Instructions:** Add EDTA to 100mL water and mix well.

###### 10 mM NiSO<sub>4</sub>, 100mL

component	Final concentration	Molecular weight (g/mol)	Amount needed
NiSO <sub>4</sub>	100mM	154.75	1.55g/100mL

**Instructions:** Add NiSO<sub>4</sub> to 100mL water and mix well.

### 4.3 Procedure

#### Wash (recommended after each run)

1. Remove the majority of the fluid in the column containing the Ni-NTA or Ni-IDA MagBeads matrix. Add 10 bv water and allow the majority of the water volume to drip out of the column.
2. Add 10 bv Wash Buffer to the column and allow the volume to completely flow through the matrix.
3. Rinse the column again with 10 bv water.
4. Finally, add 10 bv 20% (v/v) ethanol and allow the majority of the volume to drip out of the column. The MagBeads are now ready to be reused.

**Tip:** You can allow the fluid to drip through the column by gravity, or use a pressure bulb to gently force the fluid through the matrix.

#### Wash and regenerate (recommended after each run, latest after 5 runs)

1. Remove the majority of the fluid in the column containing the Ni-NTA or Ni-IDA MagBeads matrix. Add 10 bv water and allow the majority of the water volume to drip out of the column.
2. Add 10 bv 100mM EDTA to the column and allow the volume to completely flow through the matrix.
3. Rinse the column again with 10 bv water.
4. Add 10 bv Wash Buffer to the column and allow the volume to completely flow through the matrix.
5. Rinse the column with 10 bv water.
6. Add 10 bv 100mM NiSO<sub>4</sub> to recharge the matrix. Allow the volume to drip through the column by gravity.
7. Rinse the column with 10 bv water.
8. Finally, add 10 bv 20% (v/v) ethanol and allow the majority of the volume to drip out of the column. The MagBeads are now ready to be reused.

#### Wash and regenerate reduced MagBeads (e.g., after use with DTT)

1. Remove the majority of the fluid in the column containing the Ni-NTA or Ni-IDA MagBeads. Add 10 bv water and allow the majority of the water volume to drip out of the column.
2. Flush the MagBeads with 10 bv 1-3% (v/v) HCl. Minimize the exposure time of the MagBeads to HCl.
3. Rinse the column with 10 bv water.
4. If the MagBeads have not turned completely white, repeat steps 2 and 3. Otherwise, continue to step 4.
5. Add 10 bv Wash Buffer and allow the majority of the volume to drip out of the column.
6. Rinse the column with 10 bv water.
7. Add 10 bv 100mM NiSO<sub>4</sub> to recharge the MagBeads. Allow the volume to drip through the column by gravity.
8. Rinse the column with 10 bv water.
9. Finally, add 10 bv 20% (v/v) ethanol and allow the majority of the volume to drip out of the column. The MagBeads are now ready to be reused.

**Note:** MagBeads exposed to reducing agents should always be regenerated after a run.

**Note:** The concentration of HCl depends on the extent to which the MagBeads are reduced. For example, 1% HCl was sufficient to strip Ni-NTA and Ni-IDA MagBeads exposed to 1mM DTT, 2% HCl for 5mM DTT, and 3% for 10mM DTT.

## 5. technical and performance parameters

Metal ion capacity Ni-IDA MagBeads (Ni)	>15µeqv Ni <sup>2+</sup> /mL (5%-suspension)	
Metal ion capacity Ni-NTA MagBeads (Ni)	>12µeqv Ni <sup>2+</sup> /mL (5%-suspension)	
DTT stability Ni-NTA MagBeads	<10mM; robust, retains colour	
DTT stability Ni-IDA MagBeads	not recommended, turns MagBeads brown	
EDTA stability Ni-NTA MagBeads	<1.5mM; robust	
EDTA stability Ni-IDA MagBeads	not recommended	
pH stability	2-4	
chem. stability	HCl: 0.01M	SDS: 2% (Ni-IDA); not recomm. for Ni-NTA
	NaOH: 0.1M	2-Propanol
	Methanol: 100%	acetonitrile: 30% (v/v)
	Ethanol: 100%	NaOH: 1M
	Natriumacetat, pH4,0	HAc: 70%
denaturing agents	Urea: 8M	Guanidinium hydrochloride: 6M
detergents	Triton X-100: 2%	Chaps: 1%
	Tween20: 2%	
additives	Imidazol: 2M	
	Ethanol: 20% + Glycerin: 50%	EDTA: <1mM; + MgCl <sub>2</sub> : <10mM
	Na <sub>2</sub> SO <sub>4</sub> : 100mM	citrate: 60mM
	NaCl: 1.5M	citrate: 60mM; + MgCl: 80mM
red. agents	red. glutathione: 1mM	

## 6. References

Spriestersbach, A., Kubicek, J., Schaefer, F., Block, H., and Maertens, B. 2011. Purification of His-tagged Proteins. Methods Navigator.

## 7. Important Information

Genaxxon Ni-IDA and Ni-NTA MagBeads are developed, designed and sold for research purposes only. It is not to be used for human, diagnostic or drug purposes or to be administered to humans unless expressly cleared for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. All due care and attention should be exercised in the handling of many of the materials described in this manual.

## 8. Warranty

Genaxxon guarantees only for the described properties of the Ni-IDA and Ni-NTA MaBeads over a period of 2 years (for Certificate of Analysis Date) if this product is used according to the information given in this publication. However, if you are not satisfied with this product, please contact Genaxxon Bioscience GmbH using given contact form or one of its authorized distributors.

## 9. Safety information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online as pdf-file or on request ([info@genaxxon.com](mailto:info@genaxxon.com)).