GENAXXON bioscience
fon: +49(0)731 – 3608 123
fax: +49(0)731 – 3608 962
E-Mail: info@genaxxon.com

Co-IDA/ Co-NTA Agarose

Purification of His-tagged Proteins

Product	Cat#	Package size
Co-IDA Agarose 20mL 50% suspension	S5370.0010	10mL
Co-IDA Agarose 100mL 50% suspension	S5370.0050	50mL
Co-IDA Agarose 500mL 50% suspension	S5370.0250	250mL
Co-NTA Agarose 20mL 50% suspension	S5356.0010	10mL
Co-NTA Agarose 100mL 50% suspension	S5356.0050	50mL
Co-NTA Agarose 500mL 50% suspension	S5356.0250	250mL

All volume specifiation relating to the sedimented agarose resin.

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1. Overview

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.

1.1 Specifications

particle size	32-60µm
pH stability	2.0-14.0
recommended flow rate	0.5-2.0mL/min. (6.0mL/min. possible)
formulation	unbuffered suspension in 20% ethanol
binding*-/loading capacity Genaxxon Co-IDA agarose	
binding*-/loading capacity Genaxxon Co-NTA agarose	30mg of the His-tagged protein/mL agarose
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.

GENAXXON bioscience GmbH
Söflinger Str. 70 ● 89077 Ulm ● Geschäftsführer: Dr. Norbert Tröndle
Amtsgericht: 89014 Ulm ● HRB 641623
U.St.-Id.Nr. DE 220 603 767 ● Deutsche St.-Nr. 88002/33658

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E-Mail: info@genaxxon.com

2. Purification of His-tagged Proteins under native conditions using Genaxxon Co-IDA or Co-NTA Agarose

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 Co-IDA or Co-NTA Agarose resin. Reagent amounts given apply to 200mL IPTG-induced bacterial culture of a well-expressed protein (approximately 10-50mg/L). If other culture volumes are processed or protein expression is higher or lower, reagent volumes may need to be adjusted.

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 based on physical disruption (e.g., sonication or homogenization) or detergents (e.g., CHAPS) can also be used. The His-tagged target protein is purified from the cleared lysate under native conditions in a bind-wash-elute procedure. Binding is performed in batch mode (as opposed to on-column binding). This method is most efficient, especially when the target protein is present at low concentrations or the His-tag is not fully accessible. Please contact us if you have questions or need assistance optimizing a protocol for your application (info@genaxxon.com).

2.1

Equipment	Materials
☐ Ice bath ☐ Refrigerated centrifuge (min 10,000xg) ☐ Micropipettor ☐ Micropipetting tips ☐ 15mL conical centrifuge tubes ☐ Disposable gravity flow columns with capped bottom outlet, 1mL bed volume ☐ pH meter ☐ End-over-end shaker	☐ Cell pellet from a 200mL culture (approximately 0.5g) ☐ Sodium phosphate monobasic (NaH_2PO_4) ☐ Sodium chloride (NaCl) ☐ Imidazole ☐ Sodium hydroxide (NaOH) ☐ Lysozyme ☐ Benzonase® nuclease (e.g. Novagen®) ☐ Genaxxon Co-IDA Agarose (50mL; Genaxxon #S5370) or Genaxxon Co-NTA Agarose (50mL; Genaxxon #S5356) ☐ Dithiothreitol (DTT) ☐ Glycerol ☐ Sodium dodecyl sulfate (SDS) ☐ Bromophenol blue ☐ Tris base ☐ HCl ☐ Optional: Protease inhibitor cocktail without EDTA

needed but not supplied

2.2 Solutions and buffers

Lysis Buffer, 20mL

component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for stock	Stock needed for buffer
NaH_2PO_4	50mM	119.98	0.5M	29.99g/500mL	2mL
NaCl	300mM	58.44	5M	146.1g/500mL	1.2mL
Imidazole	10mM	68.08	1M	6.8g/100mL	0.2mL
Instructions: Mix in 12mL water. Adjust the pH to 8.0 using NaOH and then add water to a total volume of 20mL. 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_2PO_4	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
Instructions: 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.					

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E-Mail: info@genaxxon.com

Elution Buffer, 10mL

component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for stock	Stock needed for buffer
NaH_2PO_4	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
Instructions: Mix in 9mL 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 Co- 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/L	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.					

2.3 Procedure

1. Thaw the E. coli cell pellet on ice.
2. Resuspend the cell pellet in 10mL Lysis Buffer supplemented with 1 mg/mL lysozyme.

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

   Optional: Sonify lysate to improve cell disruption. If sonification is not feasible, freezing the cell pellet at -20°C for 30 min prior to incubation at room temperature improves lysis by lysozyme.
5. Centrifuge the lysate for 30 min at 10,000xg and 2-8°C. Collect the supernatant.

   Note: The supernatant contains the soluble proteins and is the **cleared lysate.**
6. Resuspend the Genaxxon Co-IDA or Co-NTA Agarose resin by gently inverting the bottle several times. Transfer 1mL of the resin to a 15mL conical centrifuge tube. The resin is supplied as a 50% slurry, corresponding to a 0.5mL bed volume (bv). Allow the resin to settle by gravity and remove the supernatant.
7. Add 2.5mL (5 bv) Lysis Buffer and gently resuspend the slurry to equilibrate the resin. Allow the resin to settle by gravity and remove 2mL supernatant.

   Tip: To speed up the equilibration, the resin can be pelleted at 500-1000xg.
8. Add 10mL cleared lysate to the equilibrated Genaxxon Co-IDA or Co-NTA Agarose resin and incubate at 4°C for 1h on an end-over-end shaker.

   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.
9. Transfer the binding suspension to a disposable gravity flow column with a capped bottom outlet. Use Lysis Buffer to rinse the centrifuge tube and remove resin adhered to the wall.

   Tip: Alternatively, batch binding can be performed directly in a gravity flow column with closed bottom and top outlets.
10. Remove the bottom cap of the column and collect the flowthrough.

    This is the **flow-through fraction.**
11. Wash the column with 5mL (10 bv) Wash Buffer.

    This is the **wash fraction.**

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12. Elute the His-tagged protein 5 times using 0.5mL Elution Buffer (total of 5 bv). Collect each eluate in a separate tube and determine the protein concentration of each fraction.

These are the **elution fractions.**

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.

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

3. Purification of His-tagged Proteins under denaturing conditions using Genaxxon Co-IDA or Co-NTA Agarose

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 Co-IDA or Co-NTA Agarose resin. Reagent amounts given apply to 200mL IPTG-induced bacterial culture of a well-expressed protein (approximately 10-50mg/L). If other culture volumes are processed or protein expression is higher or lower, reagent volumes may need to be adjusted.

Recombinant proteins often build insoluble so-called "inclusion bodies". These unwanted formations can be avoided by purification under denaturing conditions. For this for example urea and guanidinium salts are used within the relevant steps.

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. Binding is performed in batch mode. This method is most efficient, especially when the target protein is present at low concentrations or the His-tag is not fully accessible. Please contact us if you have questions or need assistance optimizing a protocol for your application (info@genaxxon.com).

3.1

Equipment	Materials
☐ Ice bath ☐ Refrigerated centrifuge (min 10,000xg) ☐ Micropipettor ☐ Micropipetting tips ☐ 15mL conical centrifuge tubes ☐ Disposable gravity flow columns with capped bottom outlet, mL bed volume ☐ pH meter ☐ End-over-end shaker	☐ Cell pellet from a 200mL culture (approximately 0.5g) ☐ Sodium phosphate monobasic (NaH_2PO_4) ☐ Sodium chloride (NaCl) ☐ Tris base ☐ Urea ☐ Hydrochloric acid (HCl) ☐ Sodium hydroxide (NaOH) ☐ Genaxxon Co-IDA Agarose (50mL; Genaxxon #S5370) or Genaxxon Co-NTA Agarose (50mL; Genaxxon #S5356) ☐ Dithiothreitol (DTT) ☐ Glycerol ☐ Sodium dodecyl sulfate (SDS) ☐ Bromophenol blue ☐ Optional: Benzonase® nuclease (e.g. Novagen®)

needed but not supplied

3.2 Solutions and buffers

Denaturing Lysis Buffer, pH8.0, 20mL

component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for stock	Stock needed for buffer
NaH_2PO_4	50mM	119.98	0.5M	29.99g/500mL	2mL
NaCl	300mM	58.44	5M	146.1g/500mL	1.2mL
Imidazole	10mM	68.08	1M	6.8g/100mL	0.2mL
Urea	8M	60.06	-	-	9.6g
Instructions: Dissolve in 10mL water and then bring volume to 19mL. Adjust pH to 8.0 with HCl and add water to a total volume of 20mL. Due to urea dissociation, adjust the pH immediately before use.					

GENAXXON bioscience
fon: +49(0)731 – 3608 123
fax: +49(0)731 – 3608 962
E-Mail: info@genaxxon.com

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_2PO_4	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_2PO_4	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/L	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.					

3.3 Procedure

1. Thaw the E. coli cell pellet on ice.
2. Resuspend the cell pellet in 10mL Denaturing Lysis Buffer.
3. Incubate at room temperature for 30 min on an end-overend shaker.
4. Centrifuge the lysate for 30 min at room temperature and 10,000xg. Collect the supernatant.
6. Resuspend the Genaxxon Co-IDA or Co-NTA Agarose resin by gently inverting the bottle several times. Transfer 1mL of the resin to a 15mL conical centrifuge tube. The resin is supplied as a 50% slurry, corresponding to a 0.5mL bed volume (bv). Allow the resin to settle by gravity and remove the supernatant.
7. Add the cleared lysate to the resin and incubate the mixture for 1h at room temperature on an end-over-end shaker.
8. Transfer the binding suspension to a disposable gravity flow column with a capped bottom outlet. Use Lysis Buffer to rinse the centrifuge tube and remove resin adhered to the wall.
9. Remove the bottom cap of the column and collect the flowthrough.

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: Alternatively, batch binding can be done directly in a gravity flow column with closed top and bottom outlet.

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

GENAXXON bioscience GmbH
Söflinger Str. 70 ● 89077 Ulm ● Geschäftsführer: Dr. Norbert Tröndle
Amtsgericht: 89014 Ulm ● HRB 641623
U.St.-Id.Nr. DE 220 603 767 ● Deutsche St.-Nr. 88002/33658

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10. Wash the column with 5mL (10 bv) Denaturing Wash Buffer.
11. Elute the His-tagged protein 5 times using 0.5mL Denaturing Elution Buffer (total of 5 bv). Collect each eluate in a separate tube and determine the protein concentration of each fraction.
12. 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.

This is the **elution fraction.**

This is the wash fraction.

Tip: If the target protein is acid-labile, elution can be perfored with 250-500mM 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 8M 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 trichloracetate (TCA) or similar) to remove the denaturant.
If using Benzonase® to remove nucleic acids, the concentration of urea in the Denaturing Lysis Buffer may be decreased. Benzonase® is active only at urea concentrations ≤7 M. In contrast, Gu-HCl inactivates Benzonase® even at low concentrations.

4. Washing and Regenerating Co-NTA and Co-IDA Affinity Resins

Co-NTA and Co-IDA resins should be washed after each run, and regenerated latest after 5 runs (though we recommend to regenerate the resin after each run, if possible). This protocol delineates washing and regenerating procedures for Genaxxon Co-NTA and Co-IDA Agarose, including a specific procedure for resins 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 resins loaded with other metals (e.g., Co, Fe, Al, Cu), using the appropriate solutions to recharge the resin. Please contact us if you have questions or need assistance optimizing a protocol for your application (info@genaxxon.com).

4.1

Equipment	Materials
☐ Disposable gravity flow columns (minimum 1mL bed volume)	☐ Sodium chloride (NaCl) ☐ Sodium hydroxide (NaOH) ☐ Ethylenediaminetetraacetic acid (EDTA) ☐ N,N-Dimethyldodecylamine-N-oxide (LDAO, 1g) ☐ 20% (v/v) Ethanol (C_2H_6O6) ☐ Cobalt chloride ($CoCl_2$) ☐ Hydrochloric acid (HCl)

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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/mL	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 resin being washed or regenerated.					

*LDAO is only required if a membrane protein was purified on the resin 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 resin.

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.			

100 mM $CoCl_2$, 100mL

component	Final concentration	Molecular weight (g/mol)	Amount needed
$CoCl_2$	100mM	129.84	1.298g/100mL
Instructions: Add $CoCl_2$ 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 Co-NTA or Co-IDA 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 resin is 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 Co-NTA or Co-IDA 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 $CoCl_2$ to recharge the matrix. Allow the volume to drip through the column by gravity.

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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 resin is now ready to be reused.

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

1. Remove the majority of the fluid in the column containing the Co-NTA or Co-IDA resin. Add 10 bv water and allow the majority of the water volume to drip out of the column.
2. Flush the resin with 10 bv 1-3% (v/v) HCl. Minimize the exposure time of the resin to HCl.
3. Rinse the column with 10 bv water.
4. If the resin has 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 CoCl2 to recharge the resin. 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 resin is now ready to be reused.

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

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

5. technical and performance parameters

Metal ion capacity Co-NTA agarose (Co)	>15µeqv Co^{2+}/mL	
Metal ion capacity Co-IDA agarose (Co)	>25µeqv Co^{2+}/mL	
DTT stability Co-NTA agarose	<10mM; robust, retains colour	
DTT stability Co-IDA agarose	not recommended, turns resin brown	
EDTA stability Co-NTA agarose	<1.5mM; robust	
EDTA stability Co-IDA agarose	not recommended	
pH stability	2-14	
chem. stability	HCl: 0.01M	SDS: 2% (Co-IDA); not recomm. for Co-NTA
	NaOH: 0.1M	2-Propanol
	Methanol: 100%	acetonitrile: 30% (v/v)
	Ethanol: 100%	NaOH: 1M
	Natriumacetat, pH4,0	HAc: 70%
denaturating agents	Urea: 8M	Guanidinium hydrochloride: 6M
detergents	Triton X-100: 2%	Chaps: 1%
	Tween20: 2%	
additives	Imidazol: 2M	
	Ethanol: 20% + Glycerin: 50%	EDTA: <1mM; + MgCl2: <10mM
	Na_2SO_4: 100mM	citrate: 60mM
	NaCl: 1.5M	citrate: 60mM; + MgCl: 80mM
red. agents	red. glutathione: 1mM	

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fon: +49(0)731 – 3608 123
fax: +49(0)731 – 3608 962
E-Mail: info@genaxxon.com

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 Co-IDA and Co-NTA agarose is 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 Co-IDA and Co-NTA agarose 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).

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