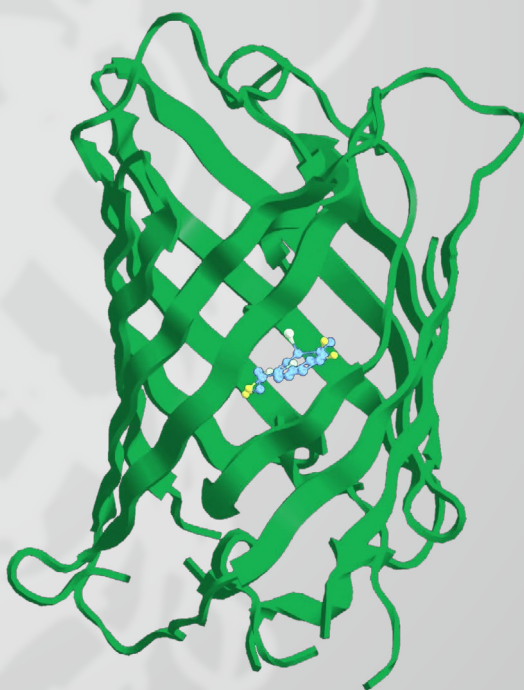


MACHEREY-NAGEL

Protein purification guide

Bioanalysis



Market leading performance for individual needs

- Save money without making compromises
- GST- and His-tag purification
- Ready to use resin and columns for any application

MACHEREY-NAGEL

www.mn-net.com



Protein purification guide

Protein purification with MACHEREY-NAGEL

The result of protein purification should be a highly pure protein sample of interest. A crucial step is the separation of the desired protein from diverse cell components and especially from other proteins. MN offers solutions for the purification of affinity-tagged proteins (such as His-tagged and GST-tagged).

Why choose MN for your protein application?

Although the purification of affinity-tagged proteins is relatively simple, the selection of best performing protocols for the optimal purification of a desired protein is not trivial. MACHEREY-NAGEL experts have years of experience in protein purification and therefore, we are a reliable partner for your protein application. Take advantage of the longterm experience and discuss your protein application with the MN research and tech support team.

Products for protein purification

Tag	Chelating ligand	Format	Product	Page	
His	NTA	Suspension	Protino® Ni-NTA Agarose	3	
		1 mL FPLC™ column	■ Protino® Ni-NTA Columns 1 mL	3	
		5 mL FPLC™ column	■ Protino® Ni-NTA Columns 5 mL	3	
		96-well plate	■ Protino® 96 Ni-NTA	4	
	TED	Bulk resin	Protino® Ni-TED Resin	5	
		Midi	■ Protino® Ni-TED 150 Packed Columns	5	
		Midi	■ Protino® Ni-TED 1000 Packed Columns	5	
	IDA	Bulk resin	Maxi	■ Protino® Ni-TED 2000 Packed Columns	5
			Bulk resin	Protino® Ni-IDA Resin	6
			Midi	■ Protino® Ni-IDA 150 Packed Columns	6
Midi		■ Protino® Ni-IDA 1000 Packed Columns	6		
GST	Glutathione	Suspension	Protino® Glutathione Agarose 4B	7	
		1 mL FPLC™ column	■ Protino® GST/4B Columns 1 mL	7	
		5 mL FPLC™ column	■ Protino® GST/4B Columns 5 mL	7	
		96-well plate	■ Protino® 96 Ni-IDA	6	
		Maxi	■ Protino® Ni-IDA 2000 Packed Columns	6	

Protein purification technology

Protino®	
Technology	IMAC (immobilized metal ion affinity chromatography) / Affinity chromatography
Chalating ligands	NTA, TED, IDA, Glutathione
Processing	Batch binding, gravity flow column chromatography, MPLC/FPLC™ applications

Icon annotation

FPLC1 Ready to use and prefilled 1 mL column for fast protein liquid chromatography (FPLC)



FPLC5 Ready to use and prefilled 5 mL column for fast protein liquid chromatography (FPLC)



96-well Mini spin columns in 96-well plate format



Resin Insoluble bead based resin matrix



Susp Suspension



Midi Midi column for gravity flow



Maxi Maxi column for gravity flow



Protein purification guide – His-tag purification

Protino® Ni-NTA

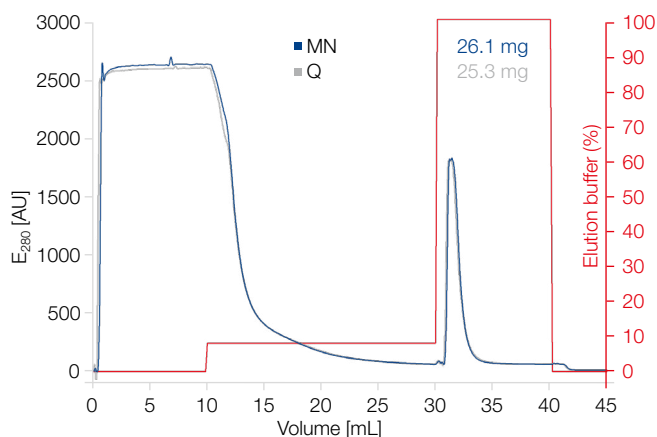
Method of choice for His-tag protein purification with best performance

- Universal use – suitable for small proteins, large protein complexes, proteins with low expression rates
- High capacity and high affinity
- Purification under native and denaturing conditions

	Susp	FPLC1	FPLC5
	Protino® Ni-NTA Agarose	Protino® Ni-NTA Columns 1 mL	Protino® Ni-NTA Columns 5 mL
Technology	IMAC (immobilized metal ion affinity chromatography)	IMAC (immobilized metal ion affinity chromatography)	IMAC (immobilized metal ion affinity chromatography)
Chelating ligand	NTA (nitrilotriacetic acid)	NTA (nitrilotriacetic acid)	NTA (nitrilotriacetic acid)
Format	Aqueous suspension (50 % (v/v), containing 30 % ethanol)	1 mL FPLC™ column	5 mL FPLC™ column
Matrix	6 % beaded agarose (crosslinked), precharged with Ni ²⁺	6 % beaded agarose (crosslinked), precharged with Ni ²⁺	6 % beaded agarose (crosslinked), precharged with Ni ²⁺
Bead size	45 – 165 µm	45 – 165 µm	45 – 165 µm
Binding capacity*	50 mg/mL*	50 mg*	250 mg*
Storage temperature	4 – 8 °C	4 – 8 °C	4 – 8 °C

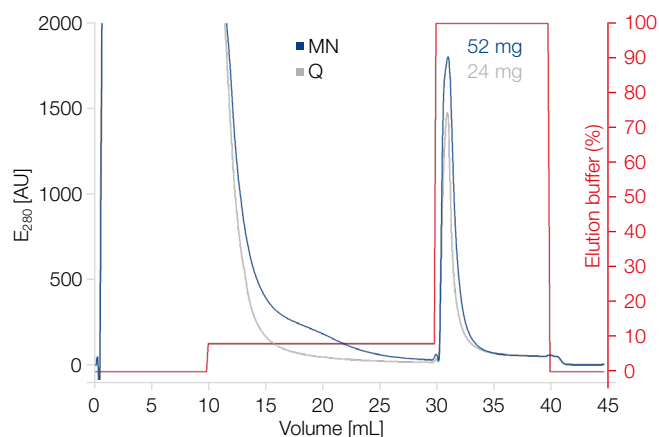
* Binding capacity will vary for each polyhistidine-tagged protein

Application data



Protino® Ni-NTA Agarose shows best performance and similar yield comparable to competitor Q's Ni-NTA Agarose

Equivalent amounts of clarified *E. coli* lysate containing His-tagged green fluorescent protein (GFP) were used for the comparison.



Higher yields for large proteins

Equivalent amounts of clarified *E. coli* lysate containing His-tagged aspartase protein were used for comparison. Superior yield of aspartase protein (208 kDa tetramer) was obtained with crosslinked agarose (Protino® Ni-NTA Columns) in comparison with highly crosslinked Superflow matrix (Q's Ni-NTA Superflow Cartridges).

Ordering information

Product	Pack of / Preps	REF
■ Protino® Ni-NTA Agarose	25 / 100 / 500 mL	745400.25 / .100 / .500
■ Protino® Ni-NTA Columns 1 mL	5	745410.5
■ Protino® Ni-NTA Columns 5 mL	1 / 5	745415.1 / .5
Related products	Pack of	REF
■ Protino® Columns 14 mL (empty gravity flow columns)	10	745250.10
■ Protino® Columns 35 mL (empty gravity flow columns)	10	745255.10

Protein purification guide – His-tag purification

Protino® 96 Ni-NTA

Method of choice for high throughput His-tag protein purification

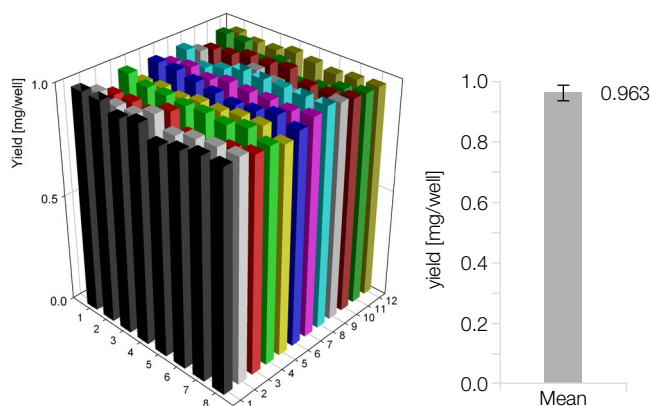
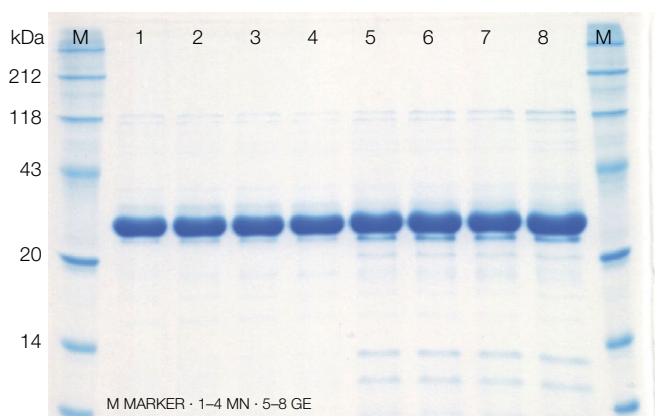
- Universal use – suitable for small proteins, large protein complexes, proteins with low expression rates
- Purification under native and denaturing conditions
- Unique Protino® Purification Plate for leak-free handling of 96 samples



Technology	IMAC (immobilized metal ion affinity chromatography)
Chelating ligand	NTA (nitrilotriacetic acid)
Format	96-well plate (suitable for vacuum or centrifugation)
Matrix	6 % beaded agarose (crosslinked), precharged with Ni ²⁺
Bead size	45 – 165 µm
Bed volume	Variable (50 µL recommended)
Reproducibility between wells	± 5 % in yield
Binding capacity*	2 mg per well (using 50 µL of settled agarose)
Storage temperature	4 – 8 °C

* Binding capacity will vary for each polyhistidine-tagged protein

Application data



High throughput purification of His-tagged green fluorescent protein (GFP) using Protino® 96 Ni-NTA (MN) in comparison to His MultiTrap™ HP (GE)

For the comparison, clarified *E. coli* lysate containing 0.5 mg His-tagged GFP was used. For binding, suspensions have been shaken for 60 min at room temperature. Pooled elution fractions were subjected to SDS-PAGE.

Well to well reliability for high throughput screenings

Protino® 96 Ni-NTA was loaded with *E. coli* lysate containing His-tagged GFP. For protein binding, suspensions were shaken for 20 min at RT. After batch binding, the yield of eluted target protein was determined. The results show that all 96 samples are consistent from well to well. Yield per well differs by just 2.7 % (relative standard deviation) indicating high well-to-well reproducibility.

Ordering information





Product	Pack of / Preps	REF
■ Protino® 96 Ni-NTA	1 × 96 / 4 × 96	745425.1 / .4
Related products	Pack of	REF
■ Protino® Purification Plate (leakfree, suitable for vacuum or centrifugation)	1 / 4	745426.1 / .4
■ NucleoVac 96 Vacuum Manifold	1	740681
■ NucleoVac Vacuum Regulator	1	740641
■ MN Shaker Frame (shaking frame for e.g., Protino® Purification Plate)	1	740489

Protein purification guide – His-tag purification

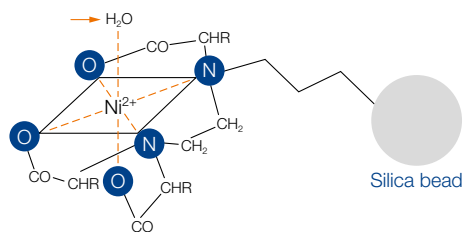
Protino® Ni-TED

The matrix of choice for highest protein purity

- Highest binding specificity – less unspecific binding of contaminating proteins compared to other common IMAC matrices
- Minimum metalion leaching due to high stability against reducing or chelating agents
- Dry resin – storage at room temperature

	 Protino® Ni-TED Resin	 Protino® Ni-TED 150 Packed Columns	 Protino® Ni-TED 1000 Packed Columns	 Protino® Ni-TED 2000 Packed Columns
Technology	IMAC (immobilized metal ion affinity chromatography)	IMAC (immobilized metal ion affinity chromatography)	IMAC (immobilized metal ion affinity chromatography)	IMAC (immobilized metal ion affinity chromatography)
Chelating ligand	TED (tris(carboxymethyl)ethylene diamine)	TED (tris(carboxymethyl)ethylene diamine)	TED (tris(carboxymethyl)ethylene diamine)	TED (tris(carboxymethyl)ethylene diamine)
Format	Bulk material	Midi gravity flow columns	Midi gravity flow columns	Maxi gravity flow columns
Matrix	Macroporous silica	Macroporous silica	Macroporous silica	Macroporous silica
Physical form	Dry matrix, precharged with Ni ²⁺	Dry matrix, precharged with Ni ²⁺	Dry matrix, precharged with Ni ²⁺	Dry matrix, precharged with Ni ²⁺
Amount of resin per column		40 mg	250 mg	500 mg
Bed volume		80 µL	500 µL	1000 µL
Binding capacity*	10 mg/g resin (5 mg/mL bed volume)	400 µg	2.5 mg	5 mg

* Binding capacity will vary for each polyhistidine-tagged protein



Scheme of a Protino® Ni-TED silica bead

5 out of 6 binding sites of Ni²⁺-ion for complexing with TED, one for selective His-tag binding (→).

- Highest specificity of Ni²⁺ to His-tagged protein
- Minimum metalion leaching
- Highest purity

Reference

Hara et al. 2018 "A chemoenzymatic process for amide bond formation by an adenylating enzyme-mediated mechanism"

Nature Scientific Reports

Ordering information






Product	Pack of / Preps	REF
■ Protino® Ni-TED Resin	5/30/120/600 g	745200.5/.30/.120/.600
■ Protino® Ni-TED 150 Packed Columns	10/50	745100.10/.50
■ Protino® Ni-TED 1000 Packed Columns	5/50	745110.5/.50
■ Protino® Ni-TED 2000 Packed Columns	5/25	745120.5/.25
Related products	Pack of	REF
■ Protino® Columns 14 mL (empty gravity flow columns)	10	745250.10
■ Protino® Columns 35 mL (empty gravity flow columns)	10	745255.10

Protein purification guide – His-tag purification

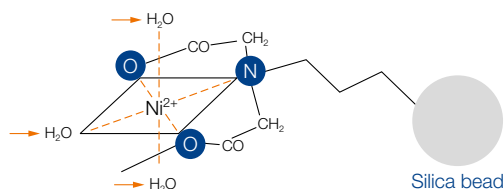
Protino® Ni-IDA

Solution for good ratio of His-tag protein yield and purity

- High protein recovery even from diluted samples due to three selective binding sites for His-tag binding
- Purification under native and denaturing conditions
- Dry resin – storage at room temperature

	 Protino® Ni-IDA Resin	 Protino® Ni-IDA 150 Packed Columns	 Protino® Ni-IDA 1000 Packed Columns	 Protino® Ni-IDA 2000 Packed Columns	 Protino® 96 Ni-IDA
Technology	IMAC (immobilized metal ion affinity chromatography)	IMAC (immobilized metal ion affinity chromatography)	IMAC (immobilized metal ion affinity chromatography)	IMAC (immobilized metal ion affinity chromatography)	IMAC (immobilized metal ion affinity chromatography)
Chelating ligand	IDA (iminodiacetic acid)	IDA (iminodiacetic acid)	IDA (iminodiacetic acid)	IDA (iminodiacetic acid)	IDA (iminodiacetic acid)
Format	Bulk material	Midi gravity flow columns	Midi gravity flow columns	Maxi gravity flow columns	96-well gravity flow plate
Matrix	Macroporous silica	Macroporous silica	Macroporous silica	Macroporous silica	Macroporous silica
Physical form	Dry matrix, precharged with Ni ²⁺	Dry matrix, precharged with Ni ²⁺	Dry matrix, precharged with Ni ²⁺	Dry matrix, precharged with Ni ²⁺	Dry matrix, precharged with Ni ²⁺
Amount of resin per column		40 mg	250 mg	500 mg	50 mg
Bed volume		80 µL	500 µL	1000 µL	100 µL
Binding capacity*	10 mg/g resin (5 mg/mL bed volume)	400 µg	2.5 mg	5 mg	1 mg/well

* Binding capacity will vary for each polyhistidine-tagged protein



Scheme of a Protino® Ni-IDA silica bead

3 out of 6 binding sites of Ni²⁺-ion for complexing with IDA, 3 for selective His-tag binding (→).

- Good protein yield and purity

Reference

Lienemann et al. 2018 "Mediator-free enzymatic electrosynthesis of formate by the Methanococcus maripaludis heterodisulfide reductase supercomplex"

Bioresource Technology

Ordering information




Product	Pack of / Preps	REF
■ Protino® Ni-IDA Resin	5/30/120/600 g	745210.5/.30/.120/.600
■ Protino® Ni-IDA 150 Packed Columns	10/50	745150.10/.50
■ Protino® Ni-IDA 1000 Packed Columns	5/50	745160.5/.50
■ Protino® Ni-IDA 2000 Packed Columns	5/25	745170.5/.25
■ Protino® 96 Ni-IDA	1 × 96/4 × 96	745300.1/.4
Related products	Pack of	REF
■ Protino® Columns 14 mL (empty gravity flow columns)	10	745250.10
■ Protino® Columns 35 mL (empty gravity flow columns)	10	745255.10

Protein purification Guide – GST-tag purification

Protino® Glutathione Agarose 4B

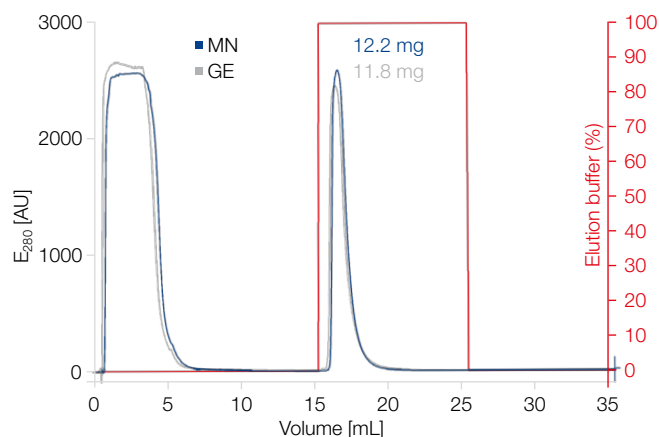
Best choice for cost effective GST-tag protein purification

- Highest performance equivalent to Glutathione Sepharose™ 4B/GSTrap™ 4B columns
- Simply replace your current products without optimization or protocol change
- Suitable for small proteins, large protein complexes, or proteins with low expression rates – universal use

	 Susp Protino® Glutathione Agarose 4B	 FPLC1 Protino® GST/4B Columns 1 mL	 FPLC5 Protino® GST/4B Columns 5 mL
Technology	Affinity chromatography	Affinity chromatography	Affinity chromatography
Chelating ligand	Glutathione, linked via sulfur atom	Glutathione, linked via sulfur atom	Glutathione, linked via sulfur atom
Format	Aqueous suspension (75 % (v/v), containing 20 % ethanol)	1 mL FPLC™ column	5 mL FPLC™ column
Matrix	4 % beaded agarose	4 % beaded agarose	4 % beaded agarose
Bead size	90 µm	90 µm	90 µm
Binding capacity*	8 mg/mL	10 mg	50 mg
Max. linear flow rate	250 cm/h	250 cm/h	250 cm/h
Max. recommended flow rate		4 mL/min	10 mL/min
Storage temperature	4–8 °C	4–8 °C	4–8 °C

* Binding capacity will vary for each GST-tagged protein

Application data



Realizing highest standards for GST-tag protein purification






Protino® GST/4B Columns (MN) 1 mL show high performance for purification of recombinant Glutathione-S-Transferase on ÄKTApri™ plus. Yield and purity are comparable to GSTrap™ 4B 1 mL columns (GE).

Ordering information

Product	Pack of / Preps	REF
■ Protino® Glutathione Agarose 4B	10 / 100 mL	745500.10 / .100
■ Protino® GST/4B Columns 1 mL	5	745510.5
■ Protino® GST/4B Columns 5 mL	1 / 5	745515.1 / .5
Related products	Pack of	REF
■ Protino® Columns 14 mL (empty gravity flow columns)	10	745250.10
■ Protino® Columns 35 mL (empty gravity flow columns)	10	745255.10

Protein purification guide

Accessory tools for protein purification

Product	Specification	REF
 ■ Protino® columns 14 mL	Gravity flow columns with filter frits to be used with Protino® Ni-TED/IDA Resin, Protino® Ni-NTA Agarose, or Protino® Glutathione Agarose 4 B	745250.10
 ■ Protino® columns 35 mL	Gravity flow columns with filter frits to be used with Protino® Ni-TED/IDA Resin, Protino® Ni-NTA Agarose, or Protino® Glutathione Agarose 4 B	745255.10
 ■ MN Frame	For optimized handling of 96-well plates with a vacuum manifold on BioRobot® 9600, 9604, and 3000 (Qiagen, MultiPROBE® II/Janus (PerkinElmer) Biomek® 2000/3000 and FX/NX (Beckman Coulter)	740680
 ■ MN Shaker Frame	Adapter frame for shaking Protino® 96-well plates	740489
 ■ Protino® Purification Plate	96-well plates with special leak-free filter frits of 20 µm pore size, purification plate for retaining chromatographic matrices (e.g., Protino® Ni-NTA, Protino® Glutathione Agarose 4 B); suitable for centrifugation and use under vacuum and positive pressure	745426.1 / .4

Ordering information

Product	Pack of / Preps	REF
His-tag purification		
■ Protino® Ni-NTA Agarose	25 / 100 / 500 mL	745400.25 / .100 / .500
■ Protino® Ni-NTA Columns 1 mL	5 × 1 mL	745410.5
■ Protino® Ni-NTA Columns 5 mL	1 × 5 mL / 5 × 5 mL	745415.1 / .5
■ Protino® 96 Ni-NTA	1 × 96 / 4 × 96	745425.1 / .4
■ Protino® Ni-TED Resin	5 / 30 / 120 / 600 g	745200.5 / .30 / .120 / .600
■ Protino® Ni-TED 150 Packed Columns	10 / 50	745100.10 / .50
■ Protino® Ni-TED 1000 Packed Columns	5 / 50	745110.5 / .50
■ Protino® Ni-TED 2000 Packed Columns	5 / 25	745120.5 / .25
■ Protino® Ni-IDA Resin	5 / 30 / 120 / 600 g	745210.5 / .30 / .120 / .600
■ Protino® Ni-IDA 150 Packed Columns	10 / 50	745150.10 / .50
■ Protino® Ni-IDA 1000 Packed Columns	5 / 50	745160.5 / .50
■ Protino® Ni-IDA 2000 Packed Columns	5 / 25	745170.5 / .25
■ Protino® 96 Ni-IDA	1 × 96 / 4 × 96	745300.1 / .4
GST-tag purification		
■ Protino® Glutathione Agarose 4B	10 / 100 mL	745500.10 / .100
■ Protino® GST/4B Columns 1 mL	5 × 1 mL	745510.5
■ Protino® GST/4B Columns 5 mL	1 × 5 mL / 5 × 5 mL	745515.1 / .5
Related products		
■ Protino® Columns 14 mL (empty gravity flow columns)	10	745250.10
■ Protino® Columns 35 mL (empty gravity flow columns)	10	745255.10
■ Protino® Purification Plate	1 / 4	745426.1 / .4
■ NucleoVac 96 Vacuum Manifold	1	740681
■ NucleoVac Vacuum Regulator	1	740641
■ MN Shaker Frame	1	740489

Trademarks: Protino® is a registered trademark of MACHEREY-NAGEL; Sepharose™, GSTrap™, FPLC™, His Multitrap™, and ÄKTAprime™ are trademarks of GE Healthcare company.

www.mn-net.com

MACHEREY-NAGEL



MACHEREY-NAGEL GmbH & Co. KG · Valencienner Str. 11 · 52355 Düren · Deutschland

DE +49 24 21 969-0 info@mn-net.com

CH +41 62 388 55 00 sales-ch@mn-net.com

FR +33 388 68 22 68 sales-fr@mn-net.com

US +1 888 321 62 24 sales-us@mn-net.com