Purification of His-tag proteins

User manual

Protino® Ni-TED 150 Packed Columns
Protino® Ni-TED 1000 Packed Columns
Protino® Ni-TED 2000 Packed Columns
Protino® Ni-TED Resin

March 2014 / Rev. 06

MACHEREY-NAGEL
www.mn-net.com

Table of contents

1 Components

1.1 Contents and storage

	Protino® Ni-TED 150 Packed Columns	
	10 preps	**50 preps**
REF	**745100.10**	**745100.50**
Protino® Ni-TED 150 Packed Columns	10	50
8 x LEW Buffer	5 mL	30 mL
4 x Elution Buffer	8 mL	20 mL
User manual	1	1

	Protino® Ni-TED 1000 Packed Columns	
	5 preps	**50 preps**
REF	**745110.5**	**745110.50**
Protino® Ni-TED 1000 Packed Columns	5	50
8 x LEW Buffer	30 mL	140 mL
4 x Elution Buffer	8 mL	100 mL
Plastic Washer	5	8
User manual	1	1

	Protino® Ni-TED 2000 Packed Columns	
	5 preps	**25 preps**
REF	**745120.5**	**745120.25**
Protino® Ni-TED 2000 Packed Columns	5	25
8 x LEW Buffer	30 mL	140 mL
4 x Elution Buffer	20 mL	100 mL
Plastic Washer	5	8
User manual	1	1

1.1 Kit contents *continued*

	Protino® Ni-TED Resin			
REF	**745200.5**	**745200.30**	**745200.120**	**745200.600**
Protino® Ni-TED Resin	5 g	30 g	120 g	600 g
User manual	1	1	1	1

Storage conditions

All kit components can be stored at room temperature (18–25 °C) and are stable up to one year.

1.2 Additional materials to be supplied by user

Reagents

- Lysozyme
- **Protino® Ni-TED 150 / 1000 / 2000 Packed Columns**

  Purification under native conditions: Kits already contain buffer stock solutions that have to be prepared according to the instructions, section 5.3.1.

  Purification under denaturing conditions: Denaturing Solubilization Buffer, Denaturing Elution Buffer, additional LEW Buffer (sodium phosphate, sodium chloride, urea, and imidazole). For buffer compositions refer to section 6.1.

- **Protino® Ni-TED Resin**

  Purification under native conditions: LEW Buffer, Elution Buffer (sodium phosphate, sodium chloride, imidazole). For buffer compositions refer to section 5.1.

  Purification under denaturing conditions: LEW buffer, Denaturing Solubilization Buffer, Denaturing Elution Buffer, (sodium phosphate, sodium chloride, urea, and imidazole). For buffer compositions refer to section 6.1.

Consumables

- Appropriate centrifugation/collection tubes
- Protino® Columns for gravity-flow column chromatography using Protino® Ni-TED Resin

  For column IMAC using **Protino® Ni-TED Resin** we generally recommend gravity-flow procedure. For this MACHEREY-NAGEL offers **Protino® Columns 14 mL** and **35 mL**.

  Protino® Columns are empty polypropylene columns with an inserted filter frit. Separate frits for covering the column bed are also included. Protino® Columns are available with volume capacities of 14 mL and 35 mL (see ordering information). They can be used to retain up to 1.4 g and 3.5 g of Protino® Ni-TED Resin, respectively. These maximum amounts of resin correspond to a protein binding capacity of 14 mg and 35 mg respectively (for 6 x His-GFPuv, concentration 2 mg/mL). For detailed information on binding capacity please also refer to sections 3.3 and 3.4).

Table 1: Protino® Columns to be used with Protino® Ni-TED Resin

	Volume capacity [mL]	Max. amount of Protino® Ni-TED Resin per column [g]	Protein binding capacity[1] [mg]
Protino® Columns 14 mL	14	1.4	14
Protino® Columns 35 mL	35	3.5	35

Equipment

- Appropriate centrifuge, sonicator

[1] Protein binding capacity refers to 6 x His-GFPuv.

2 Introduction

2.1 The basic principle

Protino® Ni-TED products enable fast and convenient purification of recombinant polyhistidine-tagged proteins by immobilized metal ion affinity chromatography (IMAC). Protino® Ni-TED is a dry silica-based resin precharged with Ni^{2+} ions. Binding of protein is based on the interaction between the polyhistidine tag of the recombinant protein and immobilized Ni^{2+} ions. The chelating group of Protino® Ni-TED is based on TED (tris-carboxymethyl ethylene diamine), a strong pentadentate metal chelator. TED occupies five of the six binding sites in the coordination sphere of the Ni^{2+} ion, the remaining coordination site of Ni^{2+} is available for protein binding (Figure 1). Compared to TED, other chelating groups such as NTA (nitrilotriacetic acid) have four binding sites available for the Ni^{2+} ion, the remaining two sites of Ni^{2+} are available for protein binding:

TED – (5 bonds) – Ni^{2+} – (1 bond) – Protein

NTA – (4 bonds) – Ni^{2+} – (2 bonds) – Protein

The additional chelation site of TED with Ni^{2+} minimizes metal leaching during purification and increases specificity for polyhistidine-tagged proteins. As a result target protein of excellent purity is eluted from the column.

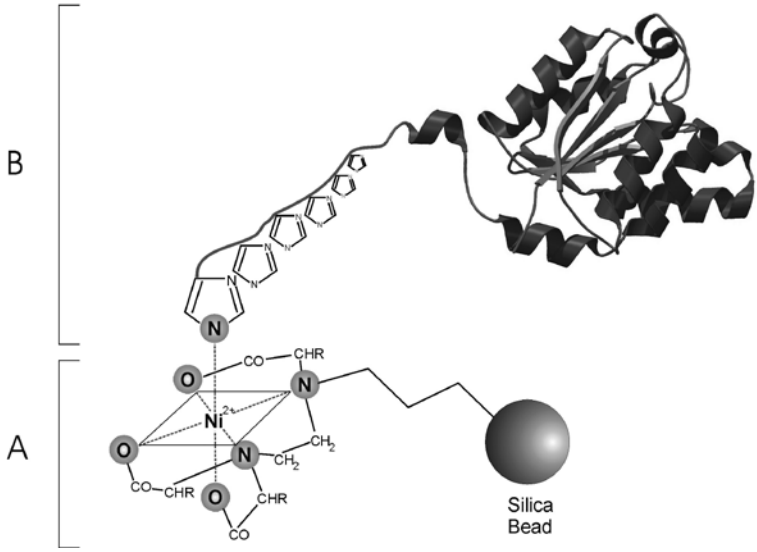

Figure 1: Binding of a polyhistidine-tagged protein to Protino® Ni-TED (schematic illustration).

A: Protino® Ni-TED a silica bead bearing the pentadentate metal chelator with bound Ni^{2+} ion. B: One histidine residue of the polyhistidine-tag of the recombinant protein binds to the resin.

2.2 About this user manual

For quick orientation in this user manual please follow the corresponding cross-reference given below.

Table 2: Protocol guide

Product	Application	Page(s)
Protino® Ni-TED Packed Columns	Gravity flow column chromatography	27, 39
Protino® Ni-TED Resin	Gravity flow column chromatography	30, 41
	Batch binding (in combination with gravity flow column chromatography)	32
	Batch purification	33
	Medium pressure column chromatography (FPLC™)	34

Protino® Ni-TED Packed Columns:

Experienced users who are performing the purification of His tagged proteins using **Protino® Ni-TED Packed Columns** may refer to the protocol-at-a-glance instead of this user manual (see section 5.3.1). The protocol-at-a-glance is designed to be used only as a supplemental tool for quick referencing while performing the purification procedure. First-time users are strongly advised to read this user manual.

The **Protino® Ni-TED Packed Columns** protocols in this manual are organized as follows: The culture volumes and volumes of the respective buffers used for a particular column size are highlighted. Each procedural step is arranged like the following example (taken from section 5.3.2):

Protino® Ni-TED Packed Columns

150	1000	2000

3 Column equilibration

Equilibrate Protino® Ni Packed Columns with **1 x LEW Buffer**. Allow the column to drain by gravity.

320 µL	2 mL	4 mL

Protino® Ni TED150/1000 Packed Columns are designed to fit into most 15 mL conical centrifuge tubes (e.g., BD Falcon REF 352097) for convenient fraction collection.

For example, if you are using **Protino® Ni-TED 150 Packed Column** you are requested to refer to the white boxes. These boxes indicate the volume of bacterial culture or buffer to be used. The respective buffer is highlighted in **bold type** within the instruction.

Referring to the a.m. example there has to be used 320 µL of LEW Buffer for column equilibration when using **Protino® Ni-TED 150 Packed Columns**.

3 Product description

3.1 Specifications

Table 3: Specifications

Specifications Protino® Ni-TED Packed Columns	150*	1000*	2000*
Protein capacity (6 x His-GFPuv, ~32 kDa)	400 µg (40 mg resin)	2.5 mg (250 mg resin)	5 mg (500 mg resin)
Application	Gravity flow columns		
Physical form	Ready-to-use columns, filled with dry matrix precharged with Ni^{2+}		
Specifications Protino® Ni-TED Resin			
Protein capacity (6 x His-GFPuv, ~32 kDa)	10 mg/g resin 5 mg/mL bed volume		
Application	Batch Gravity flow column FPLC™		
Physical form	Dry matrix, precharged with Ni^{2+}		
Max. pressure	145 psi (10 bar)		
Specifications Protino® Ni-TED Packed Columns and Resin			
Matrix	Macroporous silica		
Density	0.5 g/mL (1 g resin corresponds to 2 mL bed volume)		
Chelating group	TED (tris-carboxymethyl ethylene diamine)		
Mean particle soze	90 µm		
pH stability	Since silica is susceptible to hydrolysis at high pH, buffers with pH > 8.4 should not be used		
Storage	≤ 25 °C		

This table continues on the next page.

* The nomenclature of the Protino® Packed Columns is independent from the binding capacity but is to reflect the dimension of the column such as **small**, **medium**, and **large**.

Table 3: Specifications	
Specifications Protino® Ni-TED Packed Columns and Resin	
Recommended imidazole concentration for load/wash	0 mM
Recommended imidazole concentration for elution	≤ 250 mM

- Protino® Ni-TED products enable routine purification of recombinant polyhistidine-tagged proteins under native or denaturing conditions.
- Although designed for the purification of polyhistidine-tagged proteins from *E. coli*, Protino® Ni-TED products can also be used for the purification of polyhistidine-tagged proteins from other expression systems including insect cells, mammalian cells, and yeast.
- The capacity of Protino® Ni-TED (see Table 3) was determined by using polyhistidine-tagged green fluorescent protein (6 x His-GFPuv, ~32 kDa) expressed in *E. coli*. Capacities will vary for each His-tagged protein.

3.2 Purification under native and denaturing conditions

This manual describes methods for the preparation of cell extracts from *E. coli* and procedures for the purification of polyhistidine-tagged recombinant proteins using Protino® Ni-TED.

If recombinant proteins are expressed in *E. coli* ideally the target proteins remain soluble in the cytoplasm. However, especially proteins that are highly expressed accumulate in insoluble aggregates, which are called inclusion bodies. For solubilization of inclusion bodies buffers containing large amounts of denaturants are used. This manual includes instructions for isolation of soluble proteins (purification under native conditions, see section 5) as well as insoluble proteins from inclusion bodies (purification under denaturing conditions, see section 6).

In general for purification of polyhistidine-tagged proteins, the bacterial cells are disrupted using lysozyme in combination with sonication. After centrifugation, soluble target protein is found in the supernatant while inclusion bodies remain in the pellet. The clear supernatant can directly be subjected to further purification using Protino® Ni-TED Packed Columns or Protino® Ni-TED Resin under native conditions (see section 5). In case of massive formation of inclusion bodies the target protein is extracted from the pellet using a denaturant (8 M urea) and further purified using protocols for the purification under denaturing conditions (see section 6). If the distribution of the recombinant protein is unknown it is recommended to perform SDS-PAGE analysis using the crude cell extract prior to centrifugation and the clear supernatant after

centrifugation. While the crude cell extracts will contain both soluble and insoluble target protein, only soluble target protein is found in the supernatant.

3.3 Binding capacity of Protino® Ni-TED

3.3.1 General information

The binding capacity of Protino® Ni-TED strongly depends on the **characteristics** of the polyhistidine-tagged protein, for example amino acid composition, molecular weight, 3-D structure, oligomerization properties, etc. Furthermore, the absolute yield also depends on the total **amount** and **concentration** of the target protein in the sample which in turn directly correlate with the expression level and the cell density of the expression culture. **Therefore binding capacity will vary for each polyhistidine-tagged protein and has to be determined for each expression experiment.**

The binding behaviour of any polyhistidine-tagged protein to Protino® Ni-TED can be examined by calculating the amount of protein that is eluted as a function of the amount of protein that has been loaded (see Figure 2). Please note that the resulting graph will vary in dependence on characteristics and concentration of the individual His-tag protein. The binding curve can be divided in three stages:

1. Stage of maximum recovery. At this stage the loaded protein is bound to the resin nearly quantitatively and can be eluted nearly quantitatively, too (the binding curve is almost linear, see Figure 2, • eluted His-GFPuv).

2. Stage of increasing yield / decreasing recovery. At this stage the binding curve becomes non-linear and finally binding approaches saturation. The protein yield increases with further increasing amount of loaded protein.

3. Stage of maximum yield / minimum recovery. When loading excess protein, the available binding sites of the resin are saturated. The amount of eluted protein reaches a maximum.

The **binding capacity** for each individual protein can be defined as the yield, at which the binding curve changes from the stage of maximum recovery to the stage of increasing yield/decreasing recovery. This point is an optimal compromise between protein load and recovery and will vary for each individual protein.

3.3.2 Binding capacity

The binding capacity of Protino® Ni-TED is exemplified using the green fluorescent protein (6 x His-GFPuv, ~ 32 kDa) at a concentration of 2 mg/mL.

Please note that different recombinant proteins may show a different binding behaviour.

Figure 2 shows a plot of the amount of eluted 6 x His-GFPuv against the amount of loaded 6 x His-GFPuv. The binding curve can be divided in three stages:

1. Stage of maximum recovery: <~10 mg 6 x His-GFPuv load/g resin
2. Stage of increasing yield/decreasing recovery: >~10 mg 6 x His-GFPuv load/g resin
3. Stage of maximum yield/minimum recovery: >~60 mg 6 x His-GFPuv load/g resin

Under the above mentioned conditions the binding capacity of Protino® Ni-TED for 6 x His-GFPuv is approximately 10 mg protein per g of resin (see arrow, Figure 2). At this point the protein recovery is > 80 %. Consequently the following amounts of 6 x His-GFPuv have to be loaded: For optimal recovery: load ~ 10 mg protein per 1 g of Protino® Ni-TED Resin, for maximum yield: load ~ 60 mg protein per 1 g of Protino® Ni-TED Resin.

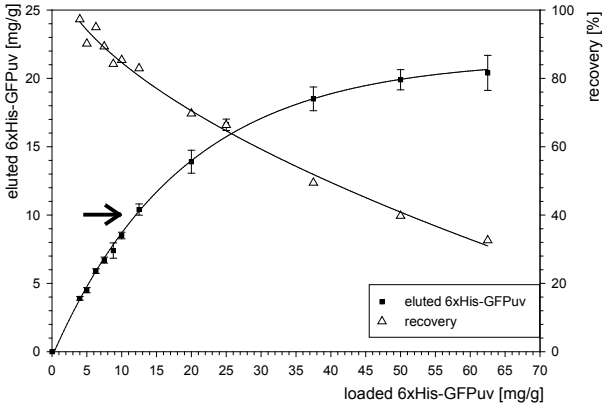

Figure 2: Binding behaviour of 6 x His-GFPuv to Protino® Ni-TED Resin

Gravity flow columns packed with 40 mg of Protino® Ni-TED Resin were loaded with increasing volumes of an *E. coli* lysate containing 6 x His-GFPuv (protein concentration 2 mg/mL). After washing with 640 µL LEW Buffer the target protein was eluted with 960 µL Elution Buffer. Yield (left axis) and recovery (right axis) of 6 x His-GFPuv are plotted versus the amount of loaded protein. For convenient analysis the values are converted to mg 6 x His-GFPuv per 1 g resin.

Recovery rates and yield can be increased by using samples containing higher concentrated polyhistidine-tagged protein (6 x His-GFPuv). Figure 3 shows that the yield of purified polyhistidine-tagged protein is not only depending on the total amount of target protein loaded on the column (also see Figure 2) but also on its concentration in the lysate. Consequently the concentration of target protein in the sample should be as high as possible.

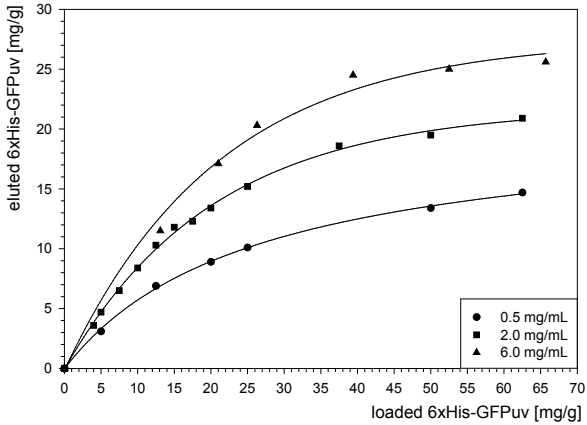

Figure 3: Binding behaviour of 6 x His-GFPuv to Protino® Ni-TED at different concentrations of the polyhistidine-tagged protein in the sample

Recombinant 6 x His-GFPuv was expressed in *E. coli*. The concentration of the target protein in the culture reached 80 mg/L. 1 g cells were lysed in 2 mL LEW Buffer according to section 3.4 in order to obtain a highly concentrated lysate. The concentration of 6 x His-GFPuv in the lysate was 6 mg/mL. Gravity flow columns packed with 40 mg of Protino® Ni-TED Resin were loaded with increasing volumes (amounts) of the lysate. After washing, the target protein was eluted with Elution buffer. The yield of 6 x His-GFPuv is plotted against the amount of loaded target protein. The same test was performed using diluted lysates with concentrations of 0.5 and 2 mg/mL. For convenient analysis the values are converted to mg 6 x His-GFPuv per 1 g resin.

Please note: The higher the protein concentration in the sample and the higher the total amount of protein loaded on Protino® Ni-TED Packed Columns or Resin, the higher will be the absolute yields. For example if loading ~ 60 mg 6 x His - GFPuv (concentration: 6 mg/mL) per g Protino® Ni-TED Resin, a maximum yield of ~ 25 mg/g can be obtained.

3.4 Culture size

As outlined above, the protein yield depends on various parameters. However, some recommendations on protein load and culture size can be given as a starting point.

Note that yield and protein load are exemplified for the 6 x His-GFPuv (~ 32 kDa) and may vary from protein to protein.

- Use rather high concentrations of the target protein in the sample.
- For **maximum yield** use an excess amount of polyhistidine-tagged protein in the loaded sample. For example apply up to 60 mg of anticipated 6 x His-GFPuv per 1 g of Protino® Ni-TED Resin.
- For **maximum recovery** use up to 10 mg of 6 x His-GFPuv per 1 g of Protino® Ni-TED Resin.

The concentration of the polyhistidine-tagged protein in the culture may vary from < 1 mg/L up to 200 mg/L depending on cell density and expression level. It is recommended to determine the protein concentration for each expression experiment, for example via SDS-PAGE. On average, 250 mL of culture will produce approximately 1 g of pelleted, wet cells.

- Transfer the cell lysate from a 100–600 mL (high expression at 100 mg/L) or 1000–6000 mL (low expression at 10 mg/L) *E. coli* culture to 1 g of Protino® Ni-TED Resin.
- In order to obtain highly concentrated lysates, lyse wet cells in 2–5 mL LEW Buffer per 1 g wet mass. The volume of LEW Buffer should be adjusted according to the amount of polyhistidine-tagged protein in the culture. For example, 1 g cells may be resuspended in 2–5 mL LEW Buffer if a protein is expressed at 50–200 mg/L. For cultures with lower target protein content 1 g cells should be resuspended in 2 mL of LEW Buffer.

For recovering polyhistidine-tagged protein from *E. coli* cultures we recommend treatment with lysozyme in combination with sonication. If you are purifying recombinant protein from eukaryotic cells, treat the cells with an appropriate buffer containing a mild detergent (Sambrook *et al.*, 1989).

Table 4: Determination of culture and buffer volume requirements					
	Concentration of HisTag protein in the culture	**Results in**	**Amount of protein load [mg]**	**Recommended *E. coli* culture volume [mL]**	**Recommended *E. coli* pellet wet mass[1] [g]**
Protino® Ni-TED Resin, 1g	high, ~100 mg/L	$Recovery_{max}$	10	100	0.4
		$Yield_{max}$	60	600	2.4
	low, ~10 mg/L	$Recovery_{max}$	10	1000	4
		$Yield_{max}$	60	6000	24
Protino® Ni-TED 150 Packed Columns (40 mg Resin)	high, ~100 mg/L	$Recovery_{max}$	0.4	4	0.02
		$Yield_{max}$	2.4	24	0.1
	low, ~10 mg/L	$Recovery_{max}$	0.4	40	0.16
		$Yield_{max}$	2.4	240	1
Protino® Ni-TED 1000 Packed Columns (250 mg Resin)	high, ~100 mg/L	$Recovery_{max}$	2.5	25	0.1
		$Yield_{max}$	15	150	0.6
	low, ~10 mg/L	$Recovery_{max}$	2.5	250	1
		$Yield_{max}$	15	1500	6
Protino® Ni-TED 2000 Packed Columns (500 mg Resin)	high, ~100 mg/L	$Recovery_{max}$	5	50	0.2
		$Yield_{max}$	30	300	1.2
	low, ~10 mg/L	$Recovery_{max}$	5	500	2
		$Yield_{max}$	30	3000	12

This table continues on the next page.

[1] On average, 250 mL of culture will produce approximately 1 g of pelleted, wet cells.

Table 4: Determination of culture and buffer volume requirements				
	Native conditions	**Denaturing conditions**		
	LEW Buffer[1] (resuspension) **[mL]**	**LEW Buffer[1]** (resuspension) **[mL]**	**LEW Buffer[1]** (washing of IB[3]) **[mL]**	**DS Buffer[2]** (lysis of IB[3]) **[mL]**
Protino® Ni-TED Resin, 1g	2	2	4	0.8
	12	12	24	4.8
	8	20	40	8
	48	120	240	48
Protino® Ni-TED 150 Packed Columns (40 mg Resin)	0.1	0.1	0.2	0.04
	0.5	0.5	1	0.2
	0.32	0.8	1.6	0.32
	2	5	10	2
Protino® Ni-TED 1000 Packed Columns (250 mg Resin)	0.5	0.5	1	0.2
	3	3	6	1.2
	2	5	10	2
	12	30	60	12
Protino® Ni-TED 2000 Packed Columns (500 mg Resin)	1	1	2	0.4
	6	6	12	2.4
	4	10	20	4
	24	60	120	24

[1] Lysis-Equilibration-Wash Buffer

[2] Denaturing Solubilization Buffer

[3] Inclusion Bodies

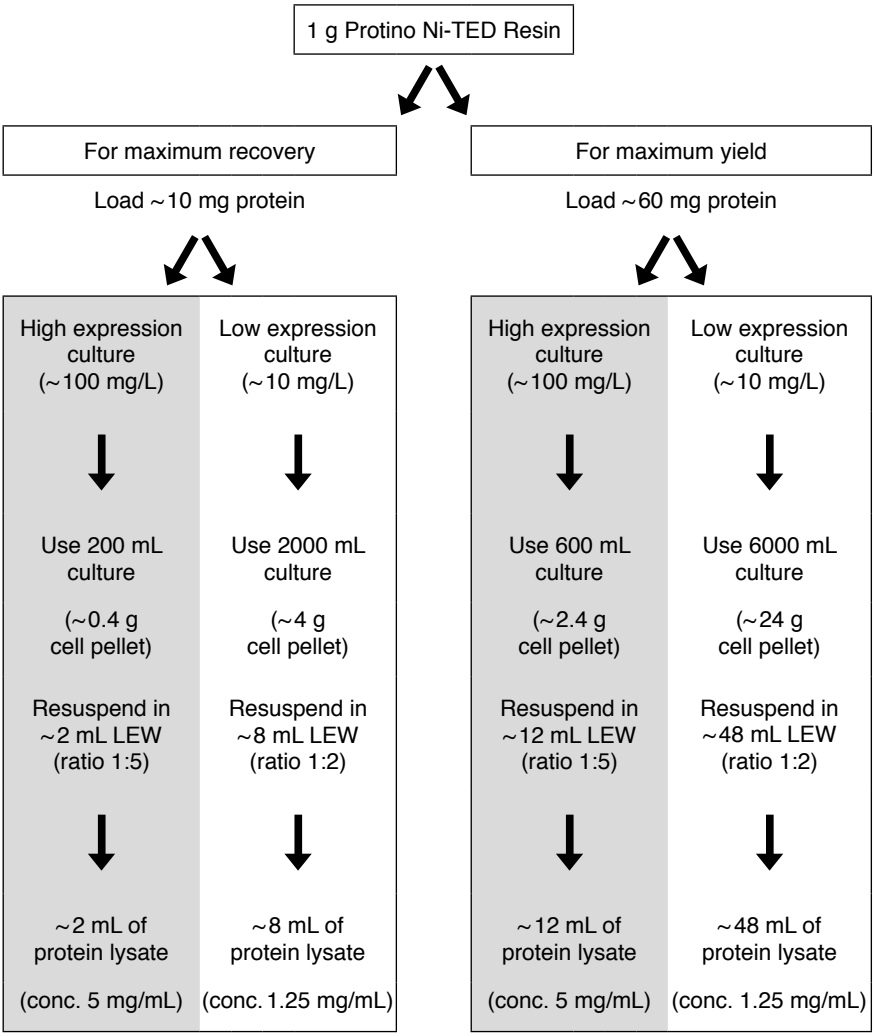

Figure 4: Required culture volumes and LEW Buffer volumes for maximum protein recovery or maximum yield in dependence on protein expression level. **The volumes noted below are only exemplary and are shown for 1 g of Protino® Ni-TED Resin (purification under native conditions).** Please use these recommendations as a starting point to evaluate optional purification results. Note that purification conditions have to be optimized for each individual polyhistidine-tagged protein.

3.5 Binding, washing, and elution

In comparison to Ni-NTA, Protino® Ni-TED is more specific for polyhistidine-tagged proteins (see Figure 5). Since virtually no contaminating host proteins bind to Protino® Ni-TED, stringent washing procedures are generally not necessary in contrast to Ni-NTA. Therefore LEW Buffer, which is used for lysis, equilibration, and washing, does not contain any imidazole. Bound polyhistidine-tagged protein can competitively be eluted by adding imidazole. The recommended Elution Buffer contains 250 mM imidazole in order to recover even strong binding, multimeric proteins with more than one polyhistidine tag (also see buffer compositions section 5.1 and 6.1). However, as shown in Figure 5, depending on the protein, elution may be equally effective in the presence of much lower imidazole concentrations. If, for example, the stability or integrity of the target protein in 250 mM imidazole is a concern the concentration of imidazole in the eluent may readily be reduced.

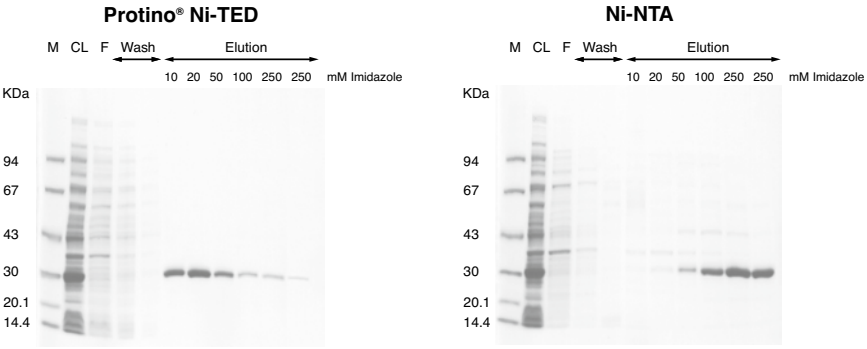

Figure 5: Purification of polyhistidine-tagged GFPuv using Protino® Ni-TED and Ni-NTA

Recombinant GFPuv was expressed in *E. coli*, lysed, loaded onto each gravity flow column, and eluted by a stepwise imidazole gradient. Eluted fractions were analyzed by SDS-PAGE. Pure polyhistidine-tagged protein can be eluted from Protino® Ni-TED (left panel) at much lower imidazole concentrations than from Ni-NTA (right panel). In addition, Ni-NTA releases contaminating proteins from 10 mM to 20 mM imidazole. Therefore, Protino® Ni-TED is more specific for polyhistidine-tagged proteins as no contaminating proteins are visible as shown for the Ni-NTA.
M = Marker proteins, CL = Cleared lysate.

3.6 Compatibility of reagents

Buffer components that chelate metal ions, such as EDTA and EGTA, should not be used since they strip Ni^{2+} ions from the matrix.

Do not use buffers with pH > 8.4, since silica dissolves in solutions of high pH.

Table 5: Reagent compatibility chart

Reagent	Effect	Comments
Sodium phosphate	Used in LEW and Elution Buffer in order to buffer the solutions at pH 8	50 mM is recommended. The pH of any buffer should be adjusted to 8, although in some cases a pH between 7 and 8 can be used
Tris	Coordinates with Ni^{2+} ions, causing a decrease in capacity	10 mM may be used, sodium phosphate buffer is recommended
Sodium Chloride	Prevents ionic interactions and therefore unspecific binding	Up to 2 M can be used, at least 0.3 M should be used
Imidazole	Binds to immobilized Ni^{2+} ions and competes with the polyhistidine-tagged proteins	Should not be included in LEW Buffer
Urea	Solubilizes protein	Use 8 M for purification under denaturing conditions
GuHCl	Solubilizes protein	Up to 6 M can be used
ß-mercaptoethanol	Prevents formation of disulfide bonds; Can reduce Ni^{2+} ions at higher concentrations	Up to 50 mM in samples has been used successfully in some cases
DTT, DTE	Can reduce Ni^{2+} ions at higher concentrations	Up to 10 mM in samples has been used successfully in some cases
Glutathione reduced	Can reduce Ni^{2+} ions at higher concentrations	Up to 30 mM in samples has been used successfully in some cases
Glycerol	Prevents hydrophobic interactions between proteins	Up to 50 % can be used

This table continues on the next page.

Table 5: Reagent compatibility chart		
EDTA	Coordinates with Ni^{2+} ions, causing a decrease in capacity at higher concentrations	Up to 10 mM in samples can be used
Ethanol	Prevents hydrophobic interactions between proteins	Up to 20 % can be used; Ethanol may precipitate proteins, causing low flow rates and column clogging
SDS	Interacts with Ni^{2+} ions, causing a decrease in capacity	Not recommended, but up to 0.2 % in samples has been used successfully in some cases
Triton, Tween	Removes background proteins	Up to 2 % can be used

4 Safety instructions

The following components of the **Protino® Ni-TED** products contain hazardous contents.

Wear gloves and goggles and follow the safety instructions given in this section.

GHS classification

Component *Inhalt*	Hazard contents *Gefahrstoff*	GHS symbol *GHS Symbol*	Hazard phrases *H-Sätze*	Precaution phrases *P-Sätze*
Protino® Ni-TED Columns / Resin	Resin with Ni in nickel(II) complexes 0.01–0.1 % *Polymer in Ni in Nickel(II)-Komplexen 0.01–0.1 %*	 Warning *Achtung*	317	261, 272, 280, 302+352, 333+313, 363

Hazard phrases

H 317 May cause an allergic skin reaction.
Kann allergische Hautreaktionen verursachen.

Precaution phrases

P 261 Avoid breathing dust.
Einatmen von Staub vermeiden.

P 272 Contaminated work clothing should not be allowed out of the workplace.
Kontaminierte Arbeitskleidung nicht außerhalb des Arbeitsplatzes tragen.

P 280 Wear protective gloves / eye protection.
Schutzhandschuhe / Augenschutz tragen.

P 302+352 IF ON SKIN: Wash with plenty of water/…
BEI KONTAKT MIT DER HAUT: Mit viel Wasser/… waschen.

P 333+313 IF skin irritation or a rash occurs: Get medical advice / attention.
Bei Hautreizung oder -ausschlag: Ärztlichen Rat einholen / ärztliche Hilfe hinzuziehen.

P 363 Wash contaminated clothing before reuse.
Kontaminierte Kleidung vor erneutem Tragen waschen.

5 Purification of polyhistidine-tagged proteins from *E. coli* under native conditions

5.1 Preparation of buffers for purification under native conditions

Protino® Ni-TED 150 / 1000 / 2000 Packed Columns *kits contain LEW/Elution Buffer stock solutions that have to be diluted according to the instructions given in the individual protocol (see sections 5.3).*

Protino® Ni-TED Resin *kits do not contain any buffers. Prepare LEW Buffer and Elution Buffer according to the instructions given in this section.*

Note that lysis buffer, equilibration buffer, and washing buffer are the same.

Note: Do not include any imidazole in the Lysis-Equilibration-Wash Buffer, since most proteins do not bind to the resin in the presence of even low imidazole concentration!

Lysis-Equilibration-Wash Buffer (1 x LEW Buffer, 1 liter):

- 50 mM NaH_2PO_4 — 7.8 g NaH_2PO_4 x 2 H_2O (MW = 156.01 g/mol)
- 300 mM NaCl — 17.5 g NaCl (MW = 58.44 g/mol)
- Adjust pH to 8.0 using NaOH

Elution Buffer (1 x buffer, 1 liter):

- 50 mM NaH_2PO_4 — 7.8 g NaH_2PO_4 x 2 H_2O (MW = 156.01 g/mol)
- 300 mM NaCl — 17.5 g NaCl (MW = 58.44 g/mol)
- 250 mM imidazole — 17.0 g imidazole (MW = 68.08 g/mol)
- Adjust pH to 8.0 using NaOH

5.2 Preparation of cleared lysates under native conditions

1 **Refer to Table 4, section 3.4 for detailed information on culture and buffer volume requirements**

Thaw the cell pellet from an *E. coli* expression culture on ice (if frozen). Resuspend **1 g of pelleted, wet cells** in **2–5 mL LEW Buffer** (for details see section 3.4). Pipette up and down, or use stirring until complete resuspension without visible cell aggregates. Perform this step on ice.

2 Add **lysozyme** to a final concentration of 1 mg/mL. Stir the solution **on ice** for **30 min**.

3 Sonicate the suspension on ice according to the instructions provided by the manufacturer (e.g., use 10 x 15 s bursts with a 15 s cooling period between each burst).

Carefully check samples´ appearance after sonication. If the lysate is still viscous from incomplete fragmentation of DNA, add 5 µg/mL DNase and stir on ice for 15 min.

4 Centrifuge the crude lysate at **10,000 x *g*** for **30 min at 4 °C** to remove cellular debris. Carefully transfer the supernatant to a clean tube without disturbing the pellet.

If the supernatant is not clear, centrifuge a second time or filter through a 0.45 µm membrane (e.g., cellulose acetate) to avoid clogging of the IMAC column with insoluble material.

Store supernatant on ice.

Proceed to section 5.3, 5.4, 5.5, 5.6, or 5.7.

5.3 Protino® Ni-TED Packed Columns – purification under native conditions

5.3.1 Protocol-at-a-glance

Note: This section only refers to Protino® Ni-TED 150, 1000, and 2000 Packed Columns.

		Protino® Ni-TED Packed Columns		
		150	**1000**	**2000**
1	**Cultivate and harvest cells**	4,500– 6,000 x *g* 15 min at 4 °C	4,500– 6,000 x *g* 15 min at 4 °C	4,500– 6,000 x *g* 15 min at 4 °C
2	**Preparation of working solutions (per column)**			
	8 x LEW Buffer	0.3 mL	1.5 mL	2.5 mL
	+ water	+ 2.1 mL	+ 10.5 mL	+ 17.5 mL
	= 1 x LEW Buffer	= 2.4 mL	= 12 mL	= 20 mL
	4 x Elution Buffer	0.25 mL	1.5 mL	3 mL
	+ water	+ 0.75 mL	+ 4.5 mL	+ 9 mL
	= 1 x Elution Buffer	= 1 mL	= 6 mL	= 12 mL
3	**Cell extract preparation** Refer to section 5.2.			
4	**Column Equilibration**			
	1 x LEW Buffer	320 µL	2 mL	4 mL
5	**Binding**	Load clarified lysate onto the column	Load clarified lysate onto the column	Load clarified lysate onto the column
6	**Washing**			
	1 x LEW Buffer	2 x 320 µL	2 x 2 mL	2 x 4 mL
7	**Elution**			
	1 x Elution Buffer	3 x 240 µL	3 x 1.5 mL	3 x 3 mL

5.3.2 Procedure

Note: Experienced users may refer to the protocol at a glance, section 5.3.1.

Protino® Ni-TED Packed Columns

150	1000	2000

1 Cultivate and harvest cells

Harvest cells from an *E. coli* expression culture by centrifugation at **4,500–6,000 x *g*** for **15 min at 4 °C**. Remove supernatant. Store cell pellet at -20 °C if not processed immediately.

2 Preparation of working solutions

Prepare 1 x LEW (Lysis/Equilibration/Wash) Buffer and 1 x Elution Buffer by diluting the supplied stock solutions.

Note: If precipitate is observed in the stock solutions, warm and shake them to dissolve precipitate prior to diluting the buffers.

Mix **8 x LEW Buffer**

0.3 mL	1.5 mL	2.5 mL

with deionized water

2.1 mL	10.5 mL	17.5 mL

to get a **final volume** of **1x LEW Buffer** sufficient for one column run.

2.4 mL	12 mL	20 mL

Mix **4 x Elution Buffer**

0.25 mL	1.5 mL	3 mL

with deionized water

0.75 mL	4.5 mL	9 mL

to get a **final volume** of **1x LEW Buffer** sufficient for one column run.

2.4 mL	6 mL	12 mL

Protino® Ni-TED Packed Columns

150	1000	2000

3 Cell Extract Preparation

Refer to section 5.2. For detailled information on culture and buffer volumes for cell extract preparation also see Table 4, section 3.4.

4 Column equilibration

Equilibrate Protino® Ni-TED Packed Columns with **1 x LEW Buffer**. Allow the column to drain by gravity.

320 µL	2 mL	4 mL

Protino® Ni-TED 150/1000 Packed Columns are designed to fit into most 15 mL conical centrifuge tubes (e.g., BD Falcon REF 352097) for convenient fraction collection.

5 Binding

Add the cleared lysate (see section 5.2) to the pre-equilibrated column and allow the column to drain by gravity.

6 Washing

Wash the column with **1 x LEW Buffer**. Allow the column to drain by gravity.

2 x 320 µL	2 x 2 mL	2 x 4 mL

7 Elution

Elute the polyhistidine-tagged protein in a new collecting tube by adding **1 x Elution Buffer**. Allow the column to drain by gravity

3 x 240 µL	3 x 1.5 mL	3 x 3 mL

*Note: Depending on protein characteristics 90 % of the eluted protein can be found in the **first** elution fraction.*

Use protein assay and / or SDS-PAGE analysis to determine which fraction(s) contain(s) the majority of the polyhistidine-tagged protein.

5.4 Protino® Ni-TED Resin – gravity-flow column chromatography under native conditions

For column IMAC using Protino® Ni-TED Resin we generally recommend gravity-flow procedure. This protocol describes gravity-flow column chromatography using **Protino® Ni-TED Resin** and **Protino® Columns** (see section 1.2).

Prepacked columns filled with 40 mg, 250 mg, or 500 mg Protino® Ni-TED Resin may readily be used (Protino® Ni-TED 150, 1000, or 2000 Packed Columns, see section 5.3 and ordering information).

Note: When using other types of chromatography columns please note that the pore size of the filter frit should be around 50 µm to ensure appropriate flow rates.

1 Column preparation

Transfer the **appropriate amount** of **Protino® Ni-TED Resin** to an empty **Protino® Column**. To achieve tight packing, gently tap the column on a hard surface until the bed height remains constant. Place a separate filter frit on top of the column bed by using a lab pen. Gently tap on the frit to ensure that there is no gap between column bed and filter frit.

1 g of Protino® Ni-TED Resin will result in 2 mL bed volume.

The amount of resin required depends on the amount of polyhistidine-tagged protein to be purified. The binding capacity of Protino® Ni-TED Resin varies from protein to protein. See section 3.3 for general guidelines.

2 Column equilibration

Equilibrate the column with **4 bed volumes** of **LEW Buffer**. Allow the column to drain by gravity.

3 Binding

Add the supernatant (cleared lysate, see section 5.2) to the pre-equilibrated column and allow the column to drain by gravity.

Apply at least 1.5 bed volumes of sample.

4 Washing

Wash the column twice with **4 bed volumes** of **LEW Buffer**. Allow the column to drain by gravity.

5 Elution

Elute the polyhistidine-tagged protein in three fractions. Add **3 x 3 bed volumes** of **Elution Buffer** and collect separately. Allow the column to drain by gravity.

Use protein assay and/or SDS-PAGE analysis to determine which fraction(s) contain(s) the majority of the polyhistidine-tagged protein.

5.5 Protino® Ni-TED Resin – batch gravity-flow purification protocol under native conditions

Although we recommend gravity flow procedure, polyhistidine-tagged proteins may be purified by the following batch / gravity-flow protocol.

Note: Usually the yield is not significantly increased using time-consuming shaking.

1 Batch binding

Add the **appropriate amount** of **Protino® Ni-TED Resin** directly to the cleared lysate (see section 5.2). Gently mix the material on an orbital shaker for 5–15 min. Do not use a magnetic stirrer to avoid generating fine particles through excessive physical force.

The amount of resin required depends on the amount of polyhistidine-tagged protein to be purified. The binding capacity of Protino® Ni-TED Resin varies from protein to protein. See section 3.3 for general guidelines.

1 g of Protino® Ni-TED Resin will result in 2 mL bed volume.

The length of time required for optimal binding will vary from protein to protein.

2 Transfer

Transfer the lysate-resin mixture to an empty chromatography column, for example Protino® Columns (see section 1.2 and ordering information). Let the resin settle by gravity flow.

3 Washing

Wash the column with **8 bed volumes** of **LEW Buffer**. Allow the column to drain by gravity.

4 Elution

Elute the polyhistidine-tagged protein in three fractions. Add **3 x 3 bed volumes** of **Elution Buffer** and collect separately. Allow the column to drain by gravity.

Use protein assay and/or SDS-PAGE analysis to determine which fraction(s) contain(s) the majority of the polyhistidine-tagged protein.

5.6 Protino® Ni-TED Resin – batch purification protocol under native conditions

Although we recommend gravity flow procedure polyhistidine-tagged proteins may be purified by the following batch protocol.

1 Batch binding

Add the **appropriate amount** of **Protino® Ni-TED Resin** directly to the cleared lysate (see section 5.2) filled in a centrifugation tube. Close the tube and mix the suspension gently, for example on an orbital shaker for 5–15 min.

The amount of resin required depends on the amount of polyhistidine-tagged protein to be purified. The binding capacity of Protino® Ni-TED Resin varies from protein to protein. See section 3.3 for general guidelines.

1 g of Protino® Ni-TED Resin will result in 2 mL bed volume.

The length of time required for optimal binding will vary from protein to protein.

Sediment the resin by gravity or centrifugation at **500 x *g*** for **1 min**. Carefully decant the supernatant and discard it.

2 Washing

Add **4 bed volumes** of **LEW Buffer** and mix for 5 min.

Sediment the resin by gravity or centrifugation at **500 x *g*** for **1 min**. Carefully decant the supernatant and dispose of it.

Repeat the washing step one or two more times (total wash **2–3 x 4 bed volumes** of **LEW Buffer**).

3 Elution

Add **3 bed volumes** of **Elution Buffer** and mix for 5 min.

Sediment the resin by gravity or centrifugation at **500 x *g*** for **1 min**.

Carefully decant or pipette the eluate in a new tube.

Repeat the elution step two more times (total elution **3 x 3 bed volumes** of **LEW Buffer**).

Use protein assay and/or SDS-PAGE analysis to determine which fraction(s) contain(s) the majority of the polyhistidine-tagged protein.

5.7 Protino® Ni-TED Resin – medium pressure column chromatography under native conditions

According to the physical stability of the Protino® Ni-TED Resin it is ideally suited for medium pressure column chromatography. The rigid matrix can be run under high flow rates and high back pressure. Furthermore Protino® Ni-TED Resin does not shrink or swell upon hydration.

As a starting point run columns at flow rates of 0.5–1.0 mL/min/cm². If the polyhistidine-tagged protein does not bind, further reduce the flow rate. Optimal flow rates have to be determined empirically, because dissociation rates vary widely from protein to protein.

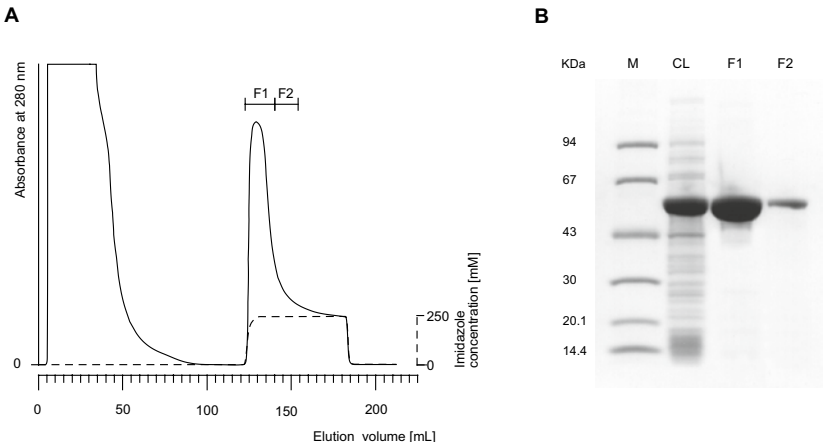

Figure 6: FPLC™ Purification with Protino® Ni-TED Resin.

Polyhistidine-tagged aspartase (~200 kDa, homo-tetramer) from *E. coli* was purified under native conditions on 10 mL Protino® Ni-TED Resin (inner diameter of the column 1.6 cm). Protein was extracted in LEW Buffer. 20 mL cleared lysate derived from 4 liters of induced *E. coli* culture was loaded at 2 mL/min. After washing the column with the same buffer at 4 mL/min protein was eluted with Elution buffer. Total yield was 120 mg.

A: Elution profile. Note that both protein and imidazole contributes to A_{280}.
To determine the step gradient profile of imidazole (dotted line) chromatography was done under identical conditions but without loading the protein sample.

B: SDS-PAGE of eluate fractions. M: Marker proteins, CL: Cleared lysate,
F1/F2: Eluted fractions

1 Column preparation – slurry packing

Make a ~10 % (w/v) slurry of **Protino® Ni-TED Resin** in degassed deionized water. Do not use a magnetic stirrer to avoid generating fine particles through excessive physical force. Slowly pour the suspension into the column. Avoid introducing air bubbles.

1 g of Protino® Ni-TED Resin will result in 2 mL bed volume.

The amount of resin required depends on the amount of polyhistidine-tagged protein to be purified. The binding capacity of Protino® Ni-TED Resin varies from protein to protein. See section 3.3 for general guidelines.

Allow the resin to settle. Insert and adjust top adapter and connect the column to the chromatography system according to the manufacturer´s instructions. Avoid introducing air bubbles.

2 Column equilibration

Equilibrate the column with **4 bed volumes** of **LEW Buffer** or until the baseline at 280 nm is stable.

3 Binding

Apply the cleared lysate (see section 5.2) to the column.

4 Washing

Wash the column with **8 bed volumes** of **LEW Buffer** or until the baseline at 280 nm is stable.

Do not add imidazole to the LEW Buffer.

5 Elution

Elute the polyhistidine-tagged protein with **5–10 bed volumes** of **Elution Buffer** using a step gradient.

When monitoring protein elution note that imidazole absorbs at 280 nm.

*Note: Depending on protein characteristics 90 % of the eluted protein can be found in the **first** elution fraction.*

Use protein assay and/or SDS-PAGE analysis to determine which fraction(s) contain(s) the majority of the polyhistidine-tagged protein.

6 Purification of polyhistidine-tagged proteins from *E. coli* under denaturing conditions

6.1 Preparation of buffers for purification under denaturating conditions

Protino® Ni-TED 150 / 1000 / 2000 Packed Columns *kits contain stock solutions of LEW Buffer and Elution Buffer for purification under native conditions. For purification under denaturing conditions prepare Denaturing Solubilization Buffer and Denaturing Elution Buffer according to the instruction given in this section. Note that additional volumes of LEW Buffer have to be prepared as well.*

Protino® Ni-TED Resin *kits do not contain any buffers. Prepare LEW Buffer, Denaturing Solubilization Buffer, and Denaturing Elution Buffer according to the instruction given in this section.*

> ***Note: Due to the dissociation of urea, prepare buffers immediately prior to use.***

Lysis-Equilibration-Wash Buffer (1 x LEW Buffer, 1 liter):

- 50 mM NaH_2PO_4 — 7.8 g NaH_2PO_4 x 2 H_2O (MW = 156.01 g/mol)
- 300 mM NaCl — 17.5 g NaCl (MW = 58.44 g/mol)
- Adjust pH to 8.0 using NaOH

Denaturing Solubilization Buffer (1 x buffer, 1 liter):

- 50 mM NaH_2PO_4 — 7.8 g NaH_2PO_4 x 2 H_2O (MW = 156.01 g/mol)
- 300 mM NaCl — 17.5 g NaCl (MW = 58.44 g/mol)
- 8 M urea — 480.5 g (MW = 60.06 g/mol)
- Adjust pH to 8.0 using NaOH

Denaturing Elution Buffer (1 x buffer, 1 liter):

- 50 mM NaH_2PO_4 — 7.8 g NaH_2PO_4 x 2 H_2O (MW = 156.01 g/mol)
- 300 mM NaCl — 17.5 g NaCl (MW = 58.44 g/mol)
- 8 M urea — 480.5 g (MW = 60.06 g/mol)
- 250 mM imidazole — 17.0 g imidazole (MW = 68.08 g/mol)
- Adjust pH to 8.0 using NaOH

6.2 Cell extract preparation under denaturing conditions

We recommend this protocol if expression leads to the formation of inclusion bodies. Cells are disrupted under native conditions using lysozyme together with sonication. After centrifugation the polyhistidine-tagged protein is extracted and solubilized from the pellet by using a denaturant (8 M urea). The extract obtained is clarified by centrifugation and applied to Protino® Ni-TED Packed Columns or Protino® Ni-TED Resin under denaturing conditions. Purification of polyhistidine-tagged proteins under denaturing conditions is similar to purification under native conditions except that the cell extract and buffers loaded on the column contain 8 M urea. For buffer compositions see section 6.1.

1 Isolation of inclusion bodies

Refer to Table 4, section 3.4 for detailed information on culture and buffer volume requirements.

Thaw the cell pellet from an *E. coli* expression culture on ice (if frozen). Resuspend **1 g** of **pelleted, wet cells** in **5 mL LEW Buffer** (without denaturant) on ice (also see section 3.4). Pipette up and down, or use stirring until complete resuspension without visible cell aggregates.

Add **lysozyme** to a final concentration of 1 mg/mL. Stir the solution **on ice** for **30 min**.

Sonicate the suspension on ice according to the instructions provided by the manufacturer (e.g., use 10 x 15 s bursts with a 15 s cooling period between each burst).

Carefully check samples´ appearance after sonication. If the lysate is still viscous from incomplete fragmentation of DNA, add 5 µg/mL DNase and stir on ice for 15 min.

Centrifuge the crude lysate at **10,000 x *g*** for **30 min at 4 °C** to collect the inclusion bodies. Discard supernatant. Keep pellet on ice.

2 Solubilization of inclusion bodies

Resuspend the pellet in **10 mL LEW Buffer** per g wet cells to wash the inclusion bodies.

Centrifuge the suspension at **10,000 x *g*** for **30 min at 4 °C**. Discard supernatant.

Resuspend the pellet in **2.0 mL Denaturing Solubilization Buffer per g wet cells** to solubilize the inclusion bodies. Homogenization or sonication may be necessary to resuspend the pellet. Dissolve the inclusion bodies by stirring on ice for 60 min.

Centrifuge at **10,000 x *g*** for **30 min at 20 °C** to remove any remaining insoluble material. Carefully transfer the supernatant to a clean tube without disturbing the pellet.

If the supernatant is not clear centrifuge a second time or filter through a 0.45 µm membrane (e.g., celluloseacetate) to avoid clogging of the IMAC column with insoluble material.

Save supernatant.

Proceed to section 6.3 or 6.4.

6.3 Protino® Ni-TED Packed Columns – purification under denaturing conditions

Protino® Ni-TED Packed Columns

150	1000	2000

1 Cell Extract Preparation

Refer to section 6.2. For detailed information on culture and buffer volumes for cell extract preparation also see Table 4, section 3.4.

2 Solubilization of inclusion bodies

Refer to section 6.2. For detailed information on culture and buffer volumes for cell extract preparation also see Table 4, section 3.4.

3 Column equilibration

Equilibrate Protino® Ni-TED Packed Columns with **Denaturing Solubilization Buffer**. Allow the column to drain by gravity.

320 µL	2 mL	4 mL

Protino® Ni-TED 150/1000 Packed Columns are designed to fit into most 15 mL conical centrifuge tubes (e.g., BD Falcon REF 352097) for convenient fraction collection.

4 Binding

Add the supernatant (solubilized protein, see section 6.2) to the pre-equilibrated column and allow the column to drain by gravity.

5 Washing

Wash the column with 8 bed volumes **Denaturing Solubilization Buffer**. Allow the column to drain by gravity.

640 µL	4 mL	8 mL

6 Elution

Elute the polyhistidine-tagged protein in a new collecting tube by adding **Denaturing Elution Buffer**. Allow the column to drain by gravity.

3 x 240 µL	3 x 1.5 mL	3 x 3 mL

Protino® Ni-TED Packed Columns

150	1000	2000

*Note: Depending on protein characteristics 90 % of the eluted protein can be found in the **first** elution fraction.*

Use protein assay and/or SDS-PAGE analysis to determine which fraction(s) contain(s) the majority of the polyhistidine-tagged protein.

6.4 Protino® Ni-TED Resin – gravity-flow column chromatography under denaturing conditions

For column IMAC using Protino® Ni-TED Resin we generally recommend the gravity-flow procedure. This protocol describes gravity-flow column chromatography using Protino® Ni-TED Resin and **Protino® Columns** (see section 1.2).

Prepacked columns filled with 40 mg, 250 mg or 500 mg Protino® Ni-TED Resin readily be used (Protino® Ni-TED 150, 1000, or 2000 Packed Columns, see section 6.3 and ordering information).

Note: When using other types of chromatography columns please note that the pore size of the filter frit should be around 50 µm to ensure appropriate flow rates.

1 Column preparation

Transfer the appropriate amount of **Protino® Ni-TED Resin** to an empty **Protino® Column**. To achieve tight packing gently tap the column on a hard surface until the bed height remains constant. Place a separate filter frit on top of the column bed by using a lab pen. Gently tap on the frit to ensure that there is no gap between column bed and filter frit.

1 g of Protino® Ni-TED Resin will result in 2 mL bed volume.

The amount of resin required depends on the amount of polyhistidine-tagged protein to be purified. The binding capacity of Protino® Ni-TED Resin varies from protein to protein. See section 3.3 for general guidelines.

2 Column equilibration

Equilibrate the column with **4 bed volumes** of **Denaturing Solubilization Buffer**. Allow the column to drain by gravity.

3 Binding

Add the supernatant (solubilized protein, see section 6.2) to the pre-equilibrated column and allow the column to drain by gravity.

4 Washing

Wash the column with **8 bed volumes** of **Denaturing Solubilization Buffer**. Allow the column to drain by gravity.

5 Elution

Elute the polyhistidine-tagged protein in three fractions. Add **3 x 3 bed volumes of Elution Buffer** and collect separately. Allow the column to drain by gravity.

*Note: Depending on protein characteristics 90 % of the eluted protein can be found in the **first** elution fraction.*

Use protein assay and/or SDS-PAGE analysis to determine which fraction(s) contain(s) the majority of the polyhistidine-tagged protein.

7 Cleaning, recharging, and storage

Cleaning

After use, wash resin with 10 bed volumes of LEW Buffer and 10 bed volumes of deionized water. Wash with 2 bed volumes of 20% ethanol and store resin in 20% ethanol at 4 °C.

Recharging

Depending on the nature of the sample Protino® Ni-TED Resin can be reused 3–5 times. Reuse should only be performed with identical polyhistidine-tagged proteins to avoid possible cross-contamination.

After the final elution step wash Protino® Ni-TED Resin with 10 bed volumes of LEW Buffer. After equilibrating with LEW Buffer the resin is ready for reuse.

Complete regeneration

If a complete regeneration is mandatory, wash resin with the following solutions:

2 bed volumes of	6 M GuHCl, 0.2 M acetic acid
5 bed volumes of	deionized water
3 bed volumes of	2% SDS
5 bed volumes of	deionized water
5 bed volumes of	100% EtOH
5 bed volumes of	deionized water
5 bed volumes of	100 mM EDTA pH 8
5 bed volumes of	deionized water
5 bed volumes of	100 mM $NiSO_4$
10 bed volumes of	deionized water

8 Appendix

8.1 Troubleshooting

Problem	Possible cause and suggestions
Sample does not enter column bed	*Sample/lysate contains insoluble material* • If the sample is not clear use centrifugation or filtration (0.45 µm membrane) to avoid clogging of the IMAC column. *Sample/lysate contains genomic DNA* • Lysate may remain viscous from incomplete shearing of genomic DNA after sonication. Add 5 µg/mL DNase and incubate on ice for 10 min.
Protein does not bind to the resin	*Problems with vector construction* • Ensure that protein and tag are in frame. *Incorrect binding conditions* • Check composition of buffers and verify pH 7–8. Ensure that there is no chelating or strong reducing reagent or imidazole present.
Protein elutes with wash buffer	*Incorrect buffer composition* • Check composition of buffers and verify pH 7–8. Ensure that there are no chelating or strong reducing agents or imidazole present.
Protein does not elute from column	*Elution conditions are too mild.* • Increase concentration of imidazole.
Unwanted proteins elute with polyhistidine-tagged protein	*Insufficient wash* • Use larger volumes for washing step. *Binding and wash conditions are too mild* • Add small amounts of imidazole (1–5 mM). Verify that the imidazole concentration is low enough to bind the polyhistidine-tagged protein.

Problem	Possible cause and suggestions
Unwanted proteins elute with polyhistidine-tagged protein *(continued)*	*Contaminating proteins and target protein are linked together via disulfide bonds* • Add up to 30 mM ß-mercaptoethanol to reduce disulfide bonds. *Contaminating proteins are proteolytic products of target protein* • Perform cell lysis at 4 °C. • Include protease inhibitors. *Expression is too low* *Contaminating host proteins have a better chance to bind to the resin when only small amounts of target protein are present in the lysate. Very low amounts of polyhistidine-tagged protein are not able to replace the majority of contaminating proteins effectively.* • Increase expression level. • Increase amount of starting cell material. • Do not exceed recommended lysis volumes.

8.2 Ordering information

Product	REF	Pack of
Protino® Ni-TED Resin	745200.5 745200.30 745200.120 745200.600	5 g 30 g 120 g 600 g
Protino® Ni-TED 150 Packed Columns (contains 40 mg of resin each)	745100.10 745100.50	10 preps 50 preps
Protino® Ni-TED 1000 Packed Columns (contains 250 mg of resin each)	745110.10 745110.50	10 preps 50 preps
Protino® Ni-TED 2000 Packed Columns (contains 500 mg of resin each)	745120.5 745120.25	5 preps 25 preps

Product	REF	Pack of
Protino® Ni-IDA Resin	745210.5 745210.30 745210.120 745210.600	5 g 30 g 120 g 600 g
Protino® Ni-IDA 150 Packed Columns (contains 40 mg of resin each)	745150.10 745150.50	10 preps 50 preps
Protino® Ni-IDA 1000 Packed Columns (contains 250 mg of resin each)	745160.10 745160.50	10 preps 50 preps
Protino® Ni-IDA 2000 Packed Columns (contains 500 mg of resin each)	745170.5 745170.25	5 preps 25 preps
Protino® Columns 14 mL (empty gravity flow columns for use with e.g. Protino® Ni-TED Resin)	745250.10	10 columns
Protino® Columns 35 mL (empty gravity flow columns for use with e.g. Protino® Ni-TED Resin)	745255.10	10 columns
NucleoBond® Rack Small (for Protino® Ni-TED/IDA 150 Packed Columns)	740562	1
NucleoBond® Rack Large (for Protino® Ni-TED/IDA 1000/2000 Packed Columns)	740563	1
Rack of MN Tube Strips (1 rack, 12 strips with 8 tubes each, and 12 Cap Strips)	740637	1 set

8.3 Product use restriction/warranty

Protino® Ni-TED products are intended, developed, designed, and sold FOR RESEARCH PURPOSES ONLY, except, however, any other function of the product being expressly described in original MACHEREY-NAGEL product leaflets.

MACHEREY-NAGEL products are intended for GENERAL LABORATORY USE ONLY! MACHEREY-NAGEL products are suited for QUALIFIED PERSONNEL ONLY! MACHEREY-NAGEL products shall in any event only be used wearing adequate PROTECTIVE CLOTHING. For detailed information please refer to the respective Material Safety Data Sheet of the product! MACHEREY-NAGEL products shall exclusively be used in an ADEQUATE TEST ENVIRONMENT. MACHEREY-NAGEL does not assume any responsibility for damages due to improper application of our products in other fields of application. Application on the human body is STRICTLY FORBIDDEN. The respective user is liable for any and all damages resulting from such application.

DNA/RNA/PROTEIN purification products of MACHEREY-NAGEL are suitable for *IN-VITRO*-USES ONLY!

ONLY MACHEREY-NAGEL products specially labeled as IVD are also suitable for *IN-VITRO*-diagnostic use. Please pay attention to the package of the product. *IN-VITRO*-diagnostic products are expressly marked as IVD on the packaging.

IF THERE IS NO IVD SIGN, THE PRODUCT SHALL NOT BE SUITABLE FOR *IN-VITRO*-DIAGNOSTIC USE!

ALL OTHER PRODUCTS NOT LABELED AS IVD ARE NOT SUITED FOR ANY CLINICAL USE (INCLUDING, BUT NOT LIMITED TO DIAGNOSTIC, THERAPEUTIC AND/OR PROGNOSTIC USE).

No claim or representations is intended for its use to identify any specific organism or for clinical use (included, but not limited to diagnostic, prognostic, therapeutic, or blood banking). It is rather in the responsibility of the user or - in any case of resale of the products - in the responsibility of the reseller to inspect and assure the use of the DNA/RNA/protein purification products of MACHEREY-NAGEL for a well-defined and specific application.

MACHEREY-NAGEL shall only be responsible for the product specifications and the performance range of MN products according to the specifications of in-house quality control, product documentation and marketing material.

This MACHEREY-NAGEL product is shipped with documentation stating specifications and other technical information. MACHEREY-NAGEL warrants to meet the stated specifications. MACHEREY-NAGEL´s sole obligation and the customer´s sole remedy is limited to replacement of products free of charge in the event products fail to perform as warranted. Supplementary reference is made to the general business terms and conditions of MACHEREY-NAGEL, which are printed on the price list. Please contact us if you wish to get an extra copy.

There is no warranty for and MACHEREY-NAGEL is not liable for damages or defects arising in shipping and handling (transport insurance for customers excluded), or out of accident or improper or abnormal use of this product; defects in products or

components not manufactured by MACHEREY-NAGEL, or damages resulting from such non-MACHEREY-NAGEL components or products.

MACHEREY-NAGEL makes no other warranty of any kind whatsoever, and SPECIFICALLY DISCLAIMS AND EXCLUDES ALL OTHER WARRANTIES OF ANY KIND OR NATURE WHATSOEVER, DIRECTLY OR INDIRECTLY, EXPRESS OR IMPLIED, INCLUDING, WITHOUT LIMITATION, AS TO THE SUITABILITY, REPRODUCTIVITY, DURABILITY, FITNESS FOR A PARTICULAR PURPOSE OR USE, MERCHANTABILITY, CONDITION, OR ANY OTHER MATTER WITH RESPECT TO MACHEREY-NAGEL PRODUCTS.

In no event shall MACHEREY-NAGEL be liable for claims for any other damages, whether direct, indirect, incidental, compensatory, foreseeable, consequential, or special (including but not limited to loss of use, revenue or profit), whether based upon warranty, contract, tort (including negligence) or strict liability arising in connection with the sale or the failure of MACHEREY-NAGEL products to perform in accordance with the stated specifications. This warranty is exclusive and MACHEREY-NAGEL makes no other warranty expressed or implied.

The warranty provided herein and the data, specifications and descriptions of this MACHEREY-NAGEL product appearing in MACHEREY-NAGEL published catalogues and product literature are MACHEREY-NAGEL´s sole representations concerning the product and warranty. No other statements or representations, written or oral, by MACHEREY-NAGEL´s employees, agent or representatives, except written statements signed by a duly authorized officer of MACHEREY-NAGEL are authorized; they should not be relied upon by the customer and are not a part of the contract of sale or of this warranty.

Product claims are subject to change. Therefore please contact our Technical Service Team for the most up-to-date information on MACHEREY-NAGEL products. You may also contact your local distributor for general scientific information. Applications mentioned in MACHEREY-NAGEL literature are provided for informational purposes only. MACHEREY-NAGEL does not warrant that all applications have been tested in MACHEREY-NAGEL laboratories using MACHEREY-NAGEL products. MACHEREY-NAGEL does not warrant the correctness of any of those applications.

Last updated: 07/2010, Rev. 03

Please contact:
MACHEREY-NAGEL GmbH & Co. KG
Tel.: +49 24 21 969-270
e-mail: tech-bio@mn-net.com

Trademarks:

FPLC is a trademark of Amersham Biosciences Corp.
Protino is a registered trademark of MACHEREY-NAGEL GmbH & Co KG