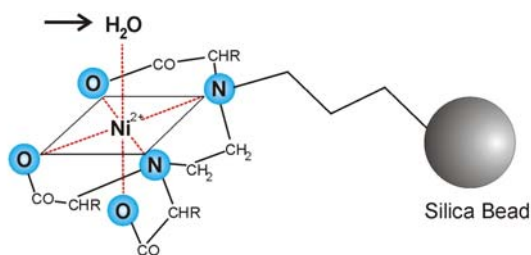


## Frequently Asked Questions - Protino® Ni-TED/IDA (Rev. 01)

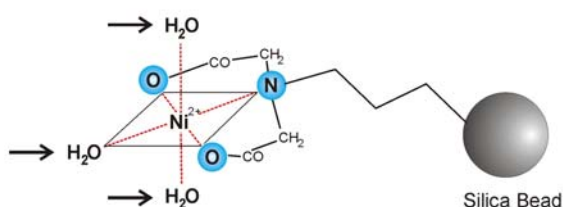
### The Product

Protino		
Matrix	macroporous silica	
Chelating group	TED	IDA
Binding sites Ni <sup>2+</sup> to His-Tag	1	3
Binding sites Chelating group to Ni <sup>2+</sup>	5	3
Ligand density	high	low

Protino® Ni-TED



Protino® Ni-IDA



	Binding Capacity* [mg]	
	TED	IDA
Protino Ni-TED/IDA 150 packed columns	0.4	0.8
Protino Ni-TED/IDA 1000 packed columns	2.5	5
Protino Ni-TED/IDA 2000 packed columns	5	10
Protino Ni-TED/IDA Resin	10 mg/g resin 5 mg/ml bed volume**	20 mg/g resin 10 mg/ml bed volume**
Protino Multi-96 Ni-IDA	/	1 mg/well

**Question**

Does the nomenclature of the Protino Ni-TED and IDA packed columns reflect the respective binding capacity?

**Answer/Recommendation**

No, the nomenclature is independent from the binding capacity but is taken to reflect the dimension of the column such as **small**, **medium**, and **large** columns.

\* refers to 6xHis-GFPuv (32 kDa)

\*\* 1 g resin corresponds to 2 ml bed volume

## The Features

Question	Answer/Recommendation		
Can Protino be used under native <b>and</b> denaturing conditions?	Yes, see user manual for recommended method of lysis		
Is Protino Resin compatible with medium pressure liquid chromatography MPLC (e.g. FPLC)?	Yes, for detailed information see user manual Protino matrix is based on silica, derived from MN Chromatography (HPLC) department. It withstands high flow rates and high pressure. In Addition it does not swell or shrink.		
Maximum pressure with HPLC/MPLC (e.g. FPLC) applications	>145 psi (>10 bar)		
Total volume capacity of Protino Ni-TED/IDA packed columns	column Protino Ni-TED/IDA	resin bed volume [ml]	residual volume [ml]
	150	0.08	1.2
	1000	0.5	3.0
	2000	1	6.9
Time per prep (Protino Ni-TED/IDA packed columns)	column Protino Ni-TED/IDA	time per prep (without lysis, approximate data) [min]	
	150	15	
	1000	30	
	2000	30	

## The Procedure

Question	Answer/Recommendation
Recommended method of lysis (Note: abbreviated protocol, for detailed information refer to user manual)	<p><b>Native</b></p> <p>Resuspend bacterial pellet in LEW buffer, stir or pipette up and down, add lysozyme (not provided), sonicate, centrifuge, load supernatant on equilibrated Protino column.</p> <p><b>Denaturing</b></p> <p>Resuspend bacterial pellet in LEW buffer, stir or pipette up and down, add lysozyme (not provided), sonicate, centrifuge, proceed with pellet, <b>dissolve inclusion bodies in Denaturing Solubilization buffer</b> (not provided, for buffer composition see user manual, section 3.6.2), centrifuge, load supernatant on equilibrated Protino column.</p>
Reuse/regeneration of Protino possible?	<p>Yes, see user manual section 7 (Storage, reuse, and regeneration of Protino)</p> <p><i>Note: reuse should only be performed with identical His-tag proteins to avoid possible cross-contaminations</i></p> <p>You may use Protino Ni-TED or IDA up to 5 times (after final elution step, wash with LEW for reuse).</p> <p>For IDA it is more effective to completely regenerate the column with 100 mM EDTA and to reload with Ni<sup>2+</sup> (for detailed procedure see user manual).</p>
Does binding capacity/efficiency change after reuse?	<p>Protino Ni-TED:</p> <ul style="list-style-type: none"> <li>- Very low reduction of capacity, material can be reused 3-5 times (between the preps, column/resin has to be washed with LEW buffer).</li> </ul> <p>Protino Ni-IDA:</p> <ul style="list-style-type: none"> <li>- High reduction of capacity already after first use of the material if column/resin is only washed with LEW buffer.</li> </ul> <p>Column/resin can be reused 3-5 times if complete regeneration is performed between the preps (wash with LEW buffer, remove Ni<sup>2+</sup>, reload with Ni<sup>2+</sup>, see user manual, section 7)</p>
Can batch binding procedures be used?	<p>Yes. Protino Resin can be added directly to the sample. The matrix will sediment very fast so that centrifugation is not necessary. Just decant and proceed with washing etc..</p>

Question	Answer/Recommendation
Can the Protino working procedure be performed at room temperature?	Yes
Can Protino products be stored at room temperature?	Yes, Protino material is a dry silica-based resin that may be stored at room temperature for up to one year
If using chromatographic columns for retaining Protino Resin, which pore size should I choose?	Protino Resin particle size is 90 µm, therefore the column should have a medium pore size of about 50 µm in order to retain the resin but allow optimal flow rates.  <i>Note: Empty Protino columns 14 ml and 35 ml are available from MN for use with Protino Ni-TED and IDA Resin</i>
May I use Protino for expression systems other than <i>E.coli</i> ?	Yes, any expression system can be used, e.g. yeast cells or mammalian cells

### The Applications

Question	Answer/Recommendation
Which Protino material is best to choose for purification of diluted samples?	Protino Ni-IDA, the binding efficiency is higher than of Protino Ni-TED
Sample: <i>E.coli</i> culture, protein with high expression rate, large sample amount. Which Protino material to choose?	Protino Ni-TED will be best choice, highest specificity
Sample: insect cells, protein with low expression rate, small sample amount. Which Protino material to choose?	Protino Ni-IDA will be best choice, higher binding capacity than Protino Ni-TED, higher specificity than Ni-NTA and Ni-IDA Agarose
How about purity of Protino Ni-TED isolated proteins?	Due to single protein binding site Protino Ni-TED shows an extremely high binding specificity, less contaminating proteins bind to the matrix compared to e.g. Protino Ni-IDA, Ni-NTA, and Ni-IDA Agarose
How about purity of Protino Ni-IDA isolated proteins?	Due to the low IDA ligand density Protino Ni-IDA allows higher purity of His Tag proteins compared to e.g. Ni-NTA and Ni-IDA Agarose

Question	Answer/Recommendation
Advantages Protino Ni-TED vs. Ni-NTA and Ni-IDA Agarose	Higher protein purity, less binding of contaminating proteins, elution of target protein at low imidazole concentrations, no imidazole necessary for washing, Protino Ni-TED is more specific for polyhistidine-tagged proteins, low metal leaching, high stability against reducing/chelating agents,  Dry material - storage at room temperature, silica base - high pressure stability, suitable for gravity flow and HPLC/MPLC applications
Advantages Protino Ni-IDA vs. Ni-NTA and Ni-IDA Agarose	Higher protein purity at low imidazole concentrations, elution of target protein at low imidazole concentrations, Protino Ni-IDA is more specific for polyhistidine-tagged proteins  Dry material - storage at room temperature, silica base - high pressure stability, suitable for gravity flow and HPLC/MPLC applications
General advantage Silica-based matrix vs Sephadex/Agarose	Dry material, storage at room temperature, compatible with high flow rates, does not shrink or swell
When should I use Protino Ni-TED?	protein of high expression rate, large sample amounts, highest purity required
When should I use Protino Ni-IDA?	protein of low expression rate, diluted samples, small sample amount, high yield required

### Reagent Compatibility

Question	Answer/Recommendation
Is Protino resistant to DTT or mercaptoethanol (reducing agents)?	Yes, up to 10 mM DTT and 50 mM ME can be used.
Is Protino resistant to EDTA (chelating agents)?	Protino Ni-TED: < 10 mM EDTA, Protino Ni-IDA: < 1 mM EDTA
Is Protino resistant to urea?	Yes, up to 8 M urea can be used.

Question	Answer/Recommendation
Is Protino resistant to SDS?	<p>SDS should be used for IMAC only with utmost care as this detergent may denature lots of proteins. Furthermore SDS is negatively charged and may potentially interact with Ni<sup>2+</sup> ions. SDS tolerance:            Protino Ni-TED: 0.2 %            Protino Ni-IDA: 0.5 %</p> <p>So if SDS is used for cell lysis it is highly recommended to use Triton X-100 instead (up to 2% can be used with both resins)</p>
Compatibility with other reagents	See reagent compatibility chart page 6
Imidazole concentration for loading	0 mM !
Imidazole concentration for elution	≤ 250 mM
Elution with pH possible?	Yes, elution can be performed with buffer of e.g. pH 4.5. <b>Note:</b> elution with imidazole is more effective

#### Protino Ni-TED/IDA - Reagent compatibility chart

Reagent	Effect	Comments
Sodium phosphate	Used in LEW and Elution buffer in order to buffer the solutions at pH 8	50 <sup>TED/IDA</sup> 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 <sup>2+</sup> ions, causing a decrease in capacity	10 <sup>TED/IDA</sup> mM may be used, sodium phosphate buffer is recommended
Sodium Chloride	Prevents ionic interactions and therefore unspecific binding	Up to 2 <sup>TED/IDA</sup> M can be used, at least 0.3 M should be used
Imidazole	Binds to immobilized Ni <sup>2+</sup> ions and competes with the polyhistidine-tagged proteins	Should not be included in LEW buffer
Urea	Solubilizes protein	Use 8 <sup>TED/IDA</sup> M for purification under denaturing conditions
GuHCl	Solubilizes protein	Up to 6 <sup>TED/IDA</sup> M can be used

**Protino Ni-TED/IDA - Reagent compatibility chart**

Reagent	Effect	Comments
$\beta$ -mercaptoethanol	Prevents formation of disulfide bonds; Can reduce Ni <sup>2+</sup> ions at higher concentrations	Up to 50 <sup>TED/IDA</sup> mM in samples has been used successfully in some cases
DTT, DTE	Can reduce Ni <sup>2+</sup> ions at higher concentrations	Up to 10 <sup>TED/IDA</sup> mM in samples has been used successfully in some cases
Glutathione reduced	Can reduce Ni <sup>2+</sup> ions at higher concentrations	Up to 30 <sup>TED/IDA</sup> mM in samples has been used successfully in some cases
Glycerol	Prevents hydrophobic interactions between proteins	Up to 50 <sup>TED/IDA</sup> % can be used.
EDTA	Coordinates with Ni <sup>2+</sup> ions, causing a decrease in capacity at higher concentrations	Up to 10 <sup>TED/1IDA</sup> mM in samples has been used successfully in some cases
Ethanol	Prevents hydrophobic interactions between proteins	Up to 20 <sup>TED/IDA</sup> % can be used; Ethanol may precipitate proteins, causing low flow rates and column clogging
SDS	Interacts with Ni <sup>2+</sup> ions, causing a decrease in capacity	Not recommended, but up to 0.2 <sup>TED</sup> /0.5 <sup>IDA</sup> % has been used successfully in some cases
Triton, Tween	Removes background proteins	Up to 2 <sup>TED/IDA</sup> % can be used

## Troubleshooting

Question	Answer/Recommendation
Sample does not enter the column bed	<p><b>Sample contains insoluble material</b> - If the sample is not clear use centrifugation or filtration (0.45 µm membrane) to remove all particulate matter and avoid clogging</p> <p><b>Sample contains genomic DNA</b> - Lysate may remain viscous from incomplete shearing of genomic DNA after sonication. Add 5 µg/ml DNase I and incubate on ice for 5 min.</p>
Target protein does not bind to the resin	<p><b>Problems with vector construction</b> - Ensure that protein and tag are in frame</p> <p><b>Incorrect buffer composition</b> - Buffer should be of pH 7-8 and should not contain imidazole, chelating agents, or strong reducing agents (see reagent compatibility chart)</p>
Target protein already elutes with wash buffer	<p><b>Incorrect buffer composition,</b> - Buffer should be of pH 7-8 and should not contain imidazole, chelating agents, or strong reducing agents (see reagent compatibility chart)</p>
Target protein is not eluted	<p><b>Elution conditions are too mild</b> - Increase imidazole concentration for elution</p>
Unwanted proteins elute with the target protein	<p><b>Insufficient wash</b> - Use larger volumes for washing step</p> <p><b>Binding and wash conditions are too mild</b> - Add traces of imidazole (TED: 1-5 mM, IDA: 1-10 mM imidazole) for washing. Verify that the imidazole concentration is low enough to keep the polyhistidine-tagged protein bound.</p> <p><b>Contaminating proteins and target protein are linked together via disulfide bonds</b> - Add up to 30 mM β-mercaptoethanol to reduce disulfide bonds</p> <p><b>Contaminating proteins are proteolytic products of target protein</b> - Perform cell lysis at 4°C - Include protease inhibitors</p>

Question	Answer/Recommendation
Unwanted proteins elute with the target protein ( <i>continued</i> )	<p><b>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 effectively replace the majority of contaminating proteins</b></p> <ul style="list-style-type: none"> <li>- Increase expression level</li> <li>- Increase amount of starting cell material</li> <li>- Do not exceed recommended lysis volumes</li> </ul>

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### Protino Ni-TED packed columns

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