Bio-Scale™ Mini Nuvia™ IMAC Ni-Charged Cartridges, 1 and 5 ml

Instruction Manual

Catalog numbers
780-0811
780-0812

BIO-RAD

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Section 1
Introduction

Bio-Scale™ Mini Nuvia™ IMAC Ni-Charged Cartridges are convenient, disposable, prepacked low-pressure chromatographic columns. Bio-Scale Mini Cartridges offer both increased run-to-run uniformity and high purity of protein through a patent pending column design and novel resin technology. Compatible with aqueous buffers most commonly used for protein purification, Bio-Scale Mini Cartridges offer improved performance for your protein separation needs.

Immobilized metal affinity chromatography (IMAC) is an excellent chromatography technique for purification of histidine-tagged proteins. The principle of IMAC is based on the affinity histidine has for metal ions. Side chains on the nitrilotriacetic acid (NTA) functional ligand selectively bind recombinant histidine-tagged proteins when the resin is charged with Ni^{2+} or other metals. The advantage of this technique is that proteins can often be purified close to homogeneity in a single step.

Bio-Scale Mini IMAC Cartridges are packed with Bio-Rad's specially designed Nuvia IMAC Ni-Charged Resin. Such characteristics as the polymeric nature, optimized ligand density, and open pore structure of the Nuvia IMAC bead result in superb mechanical strength with high stringency, low nonspecific binding, and the ability to perform separations at high flow rates.

Section 2
Product Information

Bio-Scale Mini Cartridges are disposable, easy-to-use, prepacked chromatographic cartridges supplied ready for use in convenient 1 and 5 ml sizes. Cartridges are available for a variety of chromatographic techniques, including desalting ion exchange (IEX), affinity chromatography (AC), mixed-mode, and hydrophobic interaction chromatography (HIC). Refer to **bio-rad.com** for a complete listing of items in the Bio-Scale Mini Cartridge product line.

Bio-Scale Mini Cartridges are quickly connected to liquid chromatography systems or luer syringes. The cartridges can be used with any liquid chromatography system capable of setting a high-pressure limit of 45 psi (3 bar, 0.3 MPa). Alternatively, built-in luer fittings offer convenient connection directly to a syringe for quick, one-step purification.

Nuvia IMAC Ni-Charged Resin is also available in bottles. Refer to the ordering information in Section 12 of this manual. For more information go to **bio-rad.com**.

Table 1. Bio-Scale Mini Nuvia IMAC Ni-Charged Cartridge specifications.

Sizes	**1 and 5 ml bed volumes**
Dimensions	1 ml: 40 mm length x 5.6 mm inner diameter 5 ml: 40 mm length x 12.6 mm inner diameter
Maximum pressure tolerance	45 psi
Recommended flow rates	1 ml: 1–2 ml/min (240–480 cm/hr) 5 ml: 5–10 ml/min (240–480 cm/hr)
Maximum flow rate	1 ml: 6 ml/min (1,440 cm/hr) 5 ml: 20 ml/min (963 cm/hr)
Fittings	Female luer inlet and male luer outlet
Column material	Polypropylene
Frit material	Polyethylene (HDPE)
Shipping conditions	20% ethanol
Storage recommendations	20% ethanol
Autoclavability	Not autoclavable

Table 2. Nuvia IMAC Ni-Charged Resin specifications.

Functional ligand	Nitrilotriacetic acid (NTA)
Base bead	UNOsphere™
Particle size range	38–53 µm
Mean particle size	50 µm
Metal ion capacity	≥18 µmol Cu^{2+}/ml
Dynamic binding capacity*	≥40 mg/ml
Recommended flow rate	480 cm/hr
Maximum operating pressure	45 psi
Chemical compatibility	See Table 3
Storage	4°C to ambient temperature
Storage recommendations	20% ethanol
Shelf life in 20% ethanol	>3 years at ambient temperature
Operational temperature range	4–40°C
Autoclavability	Not autoclavable

* Q10% determination of 1 mg/ml (His)6-tagged pure protein (40 kD). Note: Dynamic binding capacity will vary from protein to protein.

Nuvia IMAC Cartridges are compatible with the aqueous buffers most commonly used with IMAC purification techniques.

Table 3. Chemical compatibilities for Nuvia IMAC Ni-Charged Cartridges.*

Reagent	Stability
Chelating agent	
EDTA	1 mM
Sulfhydryl reagents	
β-mercaptoethanol	20 mM
DTT	10 mM
TCEP	20 mM
Detergents	
Nonionic (Triton, Tween)	2%
Zwitterionic (CHAPS, CHAPSO)	1%
Denaturing agents	
Guanidine-HCl	6 M
Urea	8 M
Other additives	
NaCl	2 M (include at least 300 mM NaCl in buffers)
$MgCl_2$	100 mM (use HEPES to prevent precipitation)
$CaCl_2$	5 mM (use HEPES to prevent precipitation)
Glycerol	20% (backpressure may increase significantly, slower flow rates may be required)
Ethanol	20%

* Nuvia IMAC binding capacities are unaffected up to the concentrations given when employing typical reagents used for histidine-tagged protein purification.

Section 3
Connection to Low-Pressure Chromatography Systems

Bio-Scale Mini Cartridges are ideal for use with any low-pressure chromatography system, including Bio-Rad's BioLogic™ LP System, Econo™ Gradient Pump, and Model EP-1 Econo™ Pump. For optimum performance, we recommend choosing biocompatible low-pressure tubing with an inner diameter (ID) of 1.6 mm. To order compatible polypropylene 1.6 mm barb to male and female luer end fittings, refer to the ordering information in Section 12 of this manual.

Section 4
Connection to Medium- and High-Pressure Chromatography Systems

Bio-Scale Mini Cartridges can be connected to any medium- and high-pressure liquid chromatography system set to a maximum pressure limit of 45 psi (3 bar, 0.3 MPa). Bio-Rad offers two fitting kits for easy connection of a Bio-Scale Mini Cartridge to medium- or high-pressure chromatography systems.

NGC™ Chromatography Systems and HPLC Systems

The Luer to 10-32 Adaptor Fittings Kit provides fittings necessary to connect the cartridge to nut- and ferrule-type fittings found on the NGC System and on most HPLC systems.

BioLogic DuoFlow™ Systems

The Bio-Scale Mini Cartridge to BioLogic™ System Fittings Kit includes a 1/4-28 female to male luer and 1/4-28 female to female luer to connect a Bio-Scale Mini Cartridge to Bio-Rad's BioLogic DuoFlow Chromatography System.

Fittings kit ordering information can be found in Section 12 of this manual.

Section 5
Buffers and Methods

IMAC methods can be run using either native or denaturing purification protocols. Under native conditions, proteins are purified using buffers that help retain the natural folded structure of the target protein. Under denaturing conditions, strong chaotropic agents (typically 6–8 M urea or 6 M guanidine) are added to the buffers, allowing target proteins to be purified in their unfolded states. The recommended buffer compositions and formulations are provided in the following table.

Table 4. Suggested buffer composition.

	NaCl, mM	Na Phosphate, mM	Imidazole, mM	Urea, M
Native lysis/ wash buffer 1	300	50	5	NA
Native wash buffer 2	300	50	25	NA
Native elution buffer	300	50	500	NA
Denaturing lysis/ wash buffer 1	300	50	5	6
Denaturing wash buffer 2	300	50	25	6
Denaturing elution buffer	300	50	500	6

For all buffer formulations, adjust pH to 8.0 with KOH or H_3PO_4 and filter through a 0.2 µm filter. Native buffers can be stored up to 1 year at 4–22°C; denaturing buffers must be made fresh and used within 7 days or frozen in aliquots at –20°C for later use.

Section 6
Quick Solubility Screening Protocols

Before choosing a native or denaturing purification protocol, it is useful to determine both the approximate expression level of a protein and whether the overexpressed target protein partitions into the soluble or insoluble fraction. Soluble proteins are typically purified with the native purification procedure, while insoluble proteins must be solubilized in stringent denaturants (urea or guanidine) and are purified with the denaturing procedure.

The following procedure provides a quick screen for solubility and expression level:

1. Pellet ~2 ml of *E. coli* culture by centrifugation at 4,000 x g for 10 min at 4°C.
2. Resuspend the pellet in 500 µl of PBS and sonicate on ice for 60 sec, in 10 sec pulses. Remove 50 µl of sonicate and label as the Total sample. Centrifuge the lysate at 12,000 x g for 10 min at 4°C. Transfer the supernatant to a clean tube. Remove 50 µl of the supernatant and label the tube Soluble.
3. Resuspend the insoluble pellet in 500 µl of 6 M urea in 1x PBS and sonicate on ice for 60 sec, in 10 sec pulses. Centrifuge the lysate at 12,000 x g for 10 min at 4°C. Remove 50 µl of the supernatant and label the tube Insoluble.
4. To each of the 50 µl samples, add 150 µl of Laemmli buffer and boil for 5 min at 95°C.
5. Load 10 µl of each sample on an SDS-PAGE gel.
6. Examine the soluble and insoluble fractions for the target protein. Approximate the expression level and determine partitioning of the target protein.

Section 7
Preparation of *E. coli* Lysates

For *E. coli* cultures expressing medium to high levels of histidine-tagged proteins (≥10% of total protein), 200 ml of culture will yield sufficient material for a 1 ml cartridge purification, and 1,000 ml of culture will yield sufficient material for a 5 ml cartridge purification run. For cultures expressing protein at low levels (~10% of total protein), the culture volumes will need to be determined empirically for each protein.

Native lysates

1. Harvest cell pellet by centrifugation at 8,000 x g for 10 min at 4°C.
2. Determine weight of pellet and resuspend in 10 volumes native lysis/wash buffer 1 (200 ml of culture typically yields 0.8 g of paste and results in 8 ml of lysate).
3. Sonicate the lysate on ice four times at 1 min intervals.
4. Centrifuge the lysate at 12,000 x g for 20 min at 4°C.
5. Remove the supernatant and filter it through a 0.2 µm filter immediately before applying to the cartridge.

Denatured lysates

1. Harvest cell pellet by centrifugation at 8,000 x g for 10 min at 4°C.
2. Determine weight of pellet and resuspend in 10 volumes denaturing lysis/wash buffer 1 (200 ml of culture typically yields 0.8 g of paste, and results in 8 ml of lysate).
3. Sonicate the lysate four times at 1 min intervals.
4. Centrifuge the lysate at 12,000 x g for 20 min at 4°C.
5. Remove the supernatant and filter it through a 0.2 µm filter immediately before applying to the cartridge.

Section 8
Preparing a Cartridge and Subsequent Purification

Prepare buffer sets for either the native or denaturing purification protocols using a single buffer set throughout the procedure. To prepare the cartridge for the procedure, remove the top closure and connect the cartridge to the chromatography system. Open the bottom closure and connect the cartridge outlet to the system. Flush the packing solution (20% ethanol) from the cartridge by running 2 column volumes (CV) of water at a flow rate of 2 ml/min (1 ml cartridge) or 10 ml/min (5 ml cartridge). The cartridge is now ready for the purification steps. Flow rates are given in ml/min and are specific to the 1 ml cartridge.

If using a 5 ml cartridge for a procedure, substitute the higher flow rate in the method (refer to the table below).

Table 5. Purification method suggestions.

Step	Column Volumes, CV	1 ml Cartridge Flow Rate, ml/min	5 ml Cartridge Flow Rate, ml/min
Equilibrate	5	2	10
Lysate load	Varies based on sample volume	1*	5*
Wash 1	6	1	5*
Wash 2	6	2	10
Elute	5	2	10

* Depending on sample viscosity.

Standard methods that are compatible with any type of chromatography system are listed in the following steps. To maximize binding capacity with large proteins (>100 kD), for purification at 4°C, or for purifications under denaturing conditions, the lysate load flow rate can be decreased (to 0.5 ml/min for the 1 ml cartridge and 2 ml/min for the 5 ml). Whether this decrease maximizes flow rate will have to be determined empirically for individual proteins.

1. Equilibrate the cartridge with 5 CV of equilibration/wash buffer 1 at 2 ml/min.

2. Load the sample lysate at 1 ml/min.
3. Wash the cartridge with 6 CV of wash buffer 1 at 1 ml/min.
4. Wash the cartridge with 6 CV of wash buffer 2 at 2 ml/min. **Note**: equivalent to 5% buffer B/elution buffer wash.
5. Elute the purified protein with 10 CV of elution buffer at 2 ml/min.
6. Prior to quantitation of the protein concentration, the purified protein should be exchanged into a non-imidazole buffer (imidazole can absorb at 280 nm). Purified protein from denaturing purifications should be exchanged into another buffer through dialysis.

The chromatogram in Figure 1 illustrates a representative purification of a high-expressing soluble protein purified using the native buffer set and method described in Table 5.

Note: IMAC buffers made with potassium salts are more stable than sodium salt–based buffers. However, potassium will complex with SDS in Laemmli buffer and precipitate out of solution. Prior to analyzing IMAC samples on gels, the samples must be diluted at least 1:7 with Laemmli buffer to prevent precipitation.

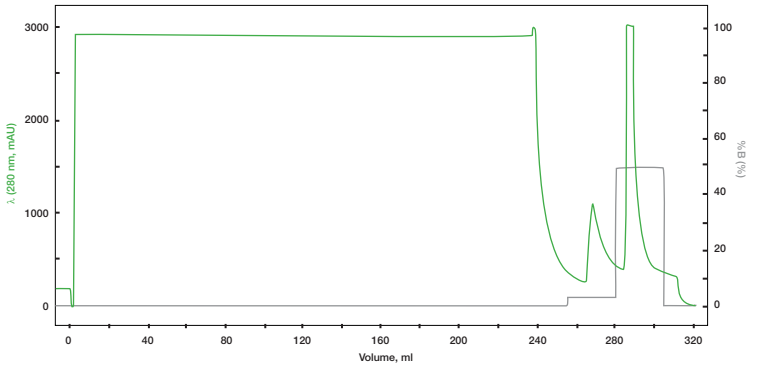

Fig. 1. Typical IMAC purification: A histidine-tagged protein was purified from the soluble fraction using the standard Nuvia IMAC native purification protocol. Clarified *E. coli* lysate was loaded onto a 5 ml Nuvia IMAC Ni-Charged Cartridge. The cartridge was washed with 6 CV of wash buffer 1 followed by 6 CV of wash buffer 2. Purified protein was eluted with 5 CV of elution buffer.

Section 9
Scaling Up

Bio-Scale Mini Cartridges are available in 1 and 5 ml cartridge formats. The Nuvia IMAC Resin is also available in various amounts, from 25 ml bottles to larger bulk quantities, for scaling up methods developed using the cartridges. For quick scale-up, two or three cartridges of the same type can be connected in series. Backpressure will increase with cartridges in series, so take care to maintain an overall system pressure ≤45 psi.

In addition, Bio-Rad carries an extensive line of empty chromatography columns from laboratory scale to process scale. Ask your local Bio-Rad representative or visit the **bio-rad.com** website.

Section 10
Regenerating, Cleaning, Sanitizing, and Storing

Protein cross-contamination, frit clogging, and increased backpressure can result from running a column beyond the recommended number of uses. After repeated use, a cartridge may run slower or produce higher backpressure, a result that should be expected due to the nature of the sample mixture. The following cleaning and regeneration procedures may be used. However, we recommend that you dispose of a cartridge after several uses. To avoid cross-contamination, we recommend that each cartridge be designated for a single protein. To maintain good flow properties, we recommend that the cartridges be cleaned between uses.

For 1 ml cartridges, run the cleaning protocol at 2 ml/min. For 5 ml cartridges, run the cleaning protocol at 5 ml/min.

High salt/acid cleaning

1. Rinse the cartridge with 2 CV water at 2 ml/min.
2. Wash the cartridge with 5 CV 500 mM NaCl, 50 mM Tris, pH 8.0 at 2 ml/min.
3. Wash the cartridge with 5 CV 500 mM NaCl, 100 mM NaOAc, pH 4.5 at 2 ml/min.
4. Rinse the cartridge with 2 CV water at 2 ml/min.
5. Store the cartridge in 20% ethanol at 4°C.

Chaotropic cleaning

1. Rinse the cartridge with 2 CV water at 2 ml/min.
2. Wash the cartridge with 5 CV 6 M guanidine HCl at 2 ml/min.
3. Rinse the cartridge with 2 CV water at 2 ml/min.
4. Store the cartridge in 20% ethanol at 4°C.

In situations where it is desirable to run different proteins over the same cartridge, completely sanitize, strip, and recharge the cartridge between sample runs. Take care when handling and disposing of metal-containing solutions.

1. Clean the cartridge with 10 CV of 1 M NaOH.
2. Rinse the cartridge with 10 CV water.
3. Strip metal ions with 5 CV of 0.1 M EDTA.
4. Rinse the cartridge with 10 CV water.
5. Recharge the cartridge with 5 CV of 0.1 M nickel sulfate pH 4.5.
6. Rinse the cartridge with 10 CV water.
7. Store the cartridge in 20% ethanol.

Section 11
Troubleshooting Guide

Cartridge clogging or slow flow rate	
Particulates in samples	Filter all samples and buffers through 0.2 µM filter prior to application
Sample too viscous	Add nuclease to lysate to degrade DNA. Centrifuge and filter lysate again
No target protein in eluent	
Low level of target	Check expression level of protein in starting SDS-PAGE material
Target protein not binding	Check levels of target protein in lysate, flowthrough, wash fractions, and eluted fractions
	Check for presence of histidine tag with antihistidine antibody
Target protein in flowthrough	
Histidine tag not accessible	Purify protein under denaturing conditions to expose histidine tag
	Reclone histidine tag onto opposite terminus (N- or C-terminus)
Proteolysis and removal	Include protease inhibitors in histidine-tag lysis buffer or purify in the cold
Precipitation during purification	
Binding capacity of cartridge exceeded	Load less sample
Protein aggregating	Include low levels of a detergent (0.1% Triton X-100, Tween 20)
	Include glycerol up to 10%
Protein too concentrated in step elution	Elute with imidazole gradient
Eluted protein is impure	
Contaminants co-eluting	Elute with imidazole gradient (10–500 mM) rather than step elution
	Increase imidazole in the wash to increase wash stringency, but keep below 40 mM
Target protein is degraded	
Proteolysis of target	Add protease inhibitors to protein lysate
	Purify at 4°C or under denaturing conditions

Section 12
Ordering Information

Bio-Scale Mini Nuvia IMAC Ni-Charged Cartridges

Catalog #	Description
780-0811	**Bio-Scale Mini Nuvia IMAC Ni-Charged Cartridge**, 1 x 5 ml column
780-0812	**Bio-Scale Mini Nuvia IMAC Ni-Charged Cartridge**, 5 x 5 ml columns

Nuvia IMAC Ni-Charged Resin

Catalog #	Description
780-0800	**Nuvia IMAC Ni-Charged Resin**, 25 ml bottle
780-0801	**Nuvia IMAC Ni-Charged Resin**, 100 ml bottle
780-0802	**Nuvia IMAC Ni-Charged Resin**, 500 ml bottle

Fittings, Tubing, and Fittings Kits

Catalog #	Description
731-8225	**1.6 mm Barb to Male Luer**, pkg of 25
731-8222	**1.6 mm Barb to Female Luer**, pkg of 25
732-0112	**Luer to 10-32 Adaptor Fittings Kit**, includes luer to polypropylene/PTFE 10-32 fittings to connect 1 cartridge to an NGC or HPLC System
732-0113	**Luer to BioLogic System Fittings Kit**, includes 1/4-28 female to male luer and 1/4-28 female to female luer to connect 1 cartridge to the BioLogic DuoFlow System

Section 13
References

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Section 14
Legal Notices

Triton is a trademark of Union Carbide. Tween is a trademark of ICI Americas, Inc.

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