Bio-Scale™ Mini Profinity™ IMAC Cartridges, 1 and 5 ml

Instruction Manual

Catalog #

732-4610

732-4612

732-4614

BIO-RAD

Table of Contents

Section 1
Introduction

Bio-Scale Mini IMAC cartridges are convenient, disposable, prepacked low-pressure chromatographic cartridges. 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 the 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 His-tagged proteins. The principle of IMAC is based on the affinity histidine has for metal ions. Side chains on the iminodiacetic acid (IDA) functional ligand selectively bind recombinant His-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 innovative Profinity™ IMAC resin. Structural characteristics such as the polymeric nature, optimized ligand density, and open pore structure of the Profinity 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

The Bio-Scale Mini cartridges are disposable, easy-to-use, prepacked chromatographic cartridges supplied ready for use in convenient 1 ml and 5 ml sizes. Cartridges are available for a variety of chromatographic techniques, including desalting ion exchange (IEX), affinity (AC), mixed-mode, and hydrophobic interaction chromatography (HIC). See Ordering Information, Section 12, for a listing of the complete Bio-Scale Mini cartridge product line.

The 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 (equivalent to 3 bar or 300 kPa). Alternatively, luer fittings offer convenient connection directly to a Luer-Lok syringe for quick, one-step purification.

Table 1 Bio-Scale Mini IMAC Cartridge Specifications

Sizes	1 ml 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 cm/hr–480 cm/hr) 5 ml:5–10 ml/min (240 cm/hr–480 cm/hr)
Maximum flow rate	1 ml: 6 ml/min (1440 cm/hr) 5 ml: 20 ml/min (963 cm/hr)
Fittings	Female luer-lock inlet and male luer-lock outlet
Column material	Polypropylene
Frit material	Polyethylene (HDPE)
Shipping conditions	20% ethanol
Storage recommendations	20% ethanol
Autoclavability	Not autoclavable

Table 2. Profinity IMAC Resin Specifications

Functional ligand	IDA
Base bead	UNOsphere
Particle size range	45–90 µm
Mean particle size	60 µm
Metal ion capacity	12–30 µmol Cu^{2+}/ml
Dynamic binding capacity*	≥15 mg/ml
Recommended linear flow rate	480 cm/hr
Maximum operating pressure	45 psi
Chemical compatibility	See table
Storage	4°C to ambient temperature
Shelf life in 20% EtOH	>1 year at ambient temperature
Operational temperature	4–40°C

* Q10% determination of 1.8 mg/ml (His)6-tagged pure protein (32 kD). Note: Dynamic binding capacity will vary from protein to protein.

Profinity IMAC cartridges are compatible with aqueous buffers most commonly used with IMAC purification techniques. For a complete list of chemical compatibilities, refer to the Profinity IMAC instruction manual, online at http://www.bio-rad.com/cmc_upload/Literature/164111/10001677B.PDF.

Table 3. Buffer and Chemical Compatibilities for Profinity IMAC cartridges

Reagent	Stability
Buffer Reagents	
Tris	50 mM
HEPES	50 mM
MOPS	50 mM
Sodium or potassium phosphate	50 mM
Chelating Agents	
EDTA, EGTA	0.1 mM
Sulfhydryl Reagents	
β–Mercaptoethanol	30 mM
DTT	5 mM
TCEP	10 mM
Detergents	
Nonionic detergents (Triton, Tween, NP-40)	5%
Cationic detergents (CTAB)	1% (care must be taken to avoid protein precipitation)
Zwitterionic detergents (CHAPS, CHAPSO)	5%
Anionic detergents (SDS, Sarkosyl)	1%
Denaturing Agents	
Guanidine-HCl	6 M
Urea	8 M
Other Additives	
NaCl	2 M (at least 300 mM NaCl should be included in buffers)
$MgCl_2$	100 mM (HEPES or Tris should be used to prevent precipitation)
$CaCl_2$	10 mM (HEPES or Tris should be used to prevent precipitation)
Glycerol	20%
Ethanol	20%
Imidazole	25 mM in wash buffer 500 mM for elution
Citrate	80 mM

* Profinity IMAC binding capacities are unaffected with typical reagents used for His-tagged protein purification, up to the concentrations given.

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.

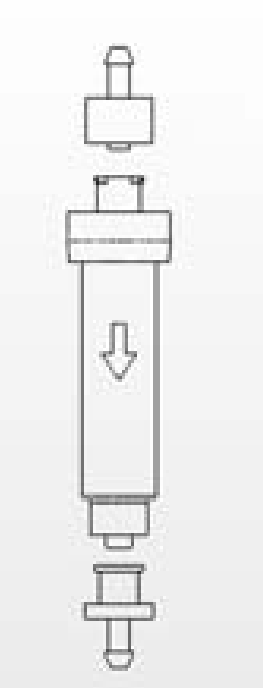


Fig. 1. Luer fittings and column: a cartridge should be mounted vertically with the arrow on the cartridge pointing downward.

To order compatible polypropylene 1.6 mm barb to male and female luer end fittings, refer to the ordering information located 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 or 300 kPa). Bio-Rad offers two fitting kits for easy connection of a Bio-Scale Mini cartridge to medium- or high-pressure chromatography 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 system.

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 most HPLC systems.

FPLC Systems

The luer to M6 adaptor fittings kit* provides fittings necessary to connect the cartridge to the M6 fittings found on FPLC or related systems.

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 chaotropes (typically 6 M urea or 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 two tables:

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

Table 4. Buffer Composition

	KCl	KH_2PO_4	Imidazole	Urea
Native lysis/ wash buffer 1	300 mM	50 mM	5 mM	N/A
Native wash buffer 2	300 mM	50 mM	10 mM	N/A
Native elution buffer	300 mM	50 mM	250 mM	N/A
Denaturing lysis/ wash buffer 1	300 mM	50 mM	5 mM	6M
Denaturing wash buffer 2	300 mM	50 mM	10 mM	6M
Denaturing elution buffer	300 mM	50 mM	250 mM	6M

Table 5. Buffer Formulations

	KCl	KH_2PO_4	Imidazole	Urea
Native lysis/wash buffer 1	22.37 g	6.80 g	0.34 g	N/A
Native wash buffer 2	22.37 g	6.80 g	0.68 g	N/A
Native elution buffer	22.37 g	6.80 g	17.02 g	N/A
Denaturing lysis/ wash buffer 1	22.37 g	6.80 g	0.34 g	360.36 g
Denaturing wash buffer 2	22.37 g	6.80 g	0.68 g	360.36 g
Denaturing elution buffer	22.37 g	6.80 g	17.02 g	360.36 g

For all buffer formulations add water to 1 L, 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

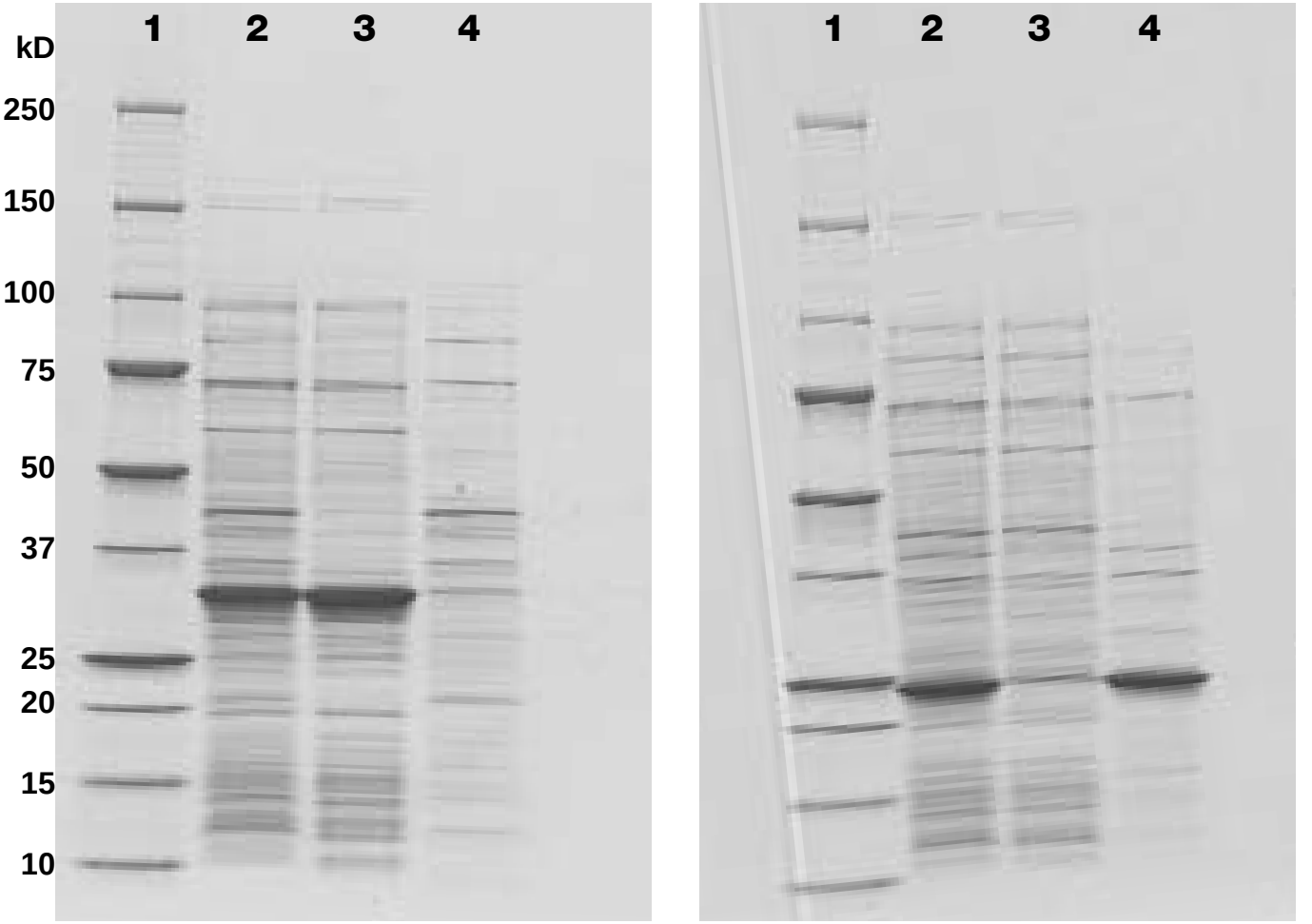
Before choosing a native or denaturing purification protocol, it is useful to determine the approximate expression level of a protein, and to determine if the overexpressed target protein partitions into the soluble or insoluble fraction. Soluble proteins are typically purified with the native purification 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 for 60 sec, on ice, in 10 sec pulses. Remove 50 µl of the 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 tube "Soluble".
3. Resuspend the insoluble pellet in 500 µl of 6 M urea in 1x PBS and sonicate for 60 sec, on ice, 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 "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.

A partitioning profile of soluble and insoluble target proteins, with approximate expression levels, can be seen in Figure 2.



~30% expression

Soluble partitioning

~25% expression

Insoluble partitioning

Fig. 2. Partioning profiles. For both gels, Precision Plus Protein™ molecular weight markers were loaded in lane 1, followed by the total, soluble, and insoluble fractions in lanes 2–4 respectively. The first panel depicts a 32 kD target protein, which partitions into the soluble fraction and can be purified using the native protocol (outlined on page 15). A representative chromatogram and gel for the purification of this target protein is shown in Fig. 5 on page 20. The second panel depicts a 24 kD target protein, which partitions into the insoluble fraction and can be purified using the denaturing protocol (outlined on page 16).

Section 7
Preparation of *E. coli* Lysates

For *E. coli* cultures expressing medium to high levels of His-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) 4 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 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 4 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 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% EtOH) 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 a 5 ml cartridge is used for a procedure, substitute the higher flow rate in the method (refer to the table below).

Table 6. Purification Method Suggestions

Step	CV	1 ml Cartridge Flow Rate	5 ml Cartridge Flow Rate
Equilibrate	5	2 ml/min	10 ml/min
Lysate Load	5 to 10	2 ml/min*	10 ml/min*
Wash 1	6	2 ml/min	10 ml/min
Wash 2	6	2 ml/min	10 ml/min
Elute	5	2 ml/min	10 ml/min

Standard methods that are compatible with any type of chromatography system are listed below. 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). This will have to be determined empirically for individual proteins.

1. Equilibrate the cartridge with 5 column volumes (CV) of equilibration/wash buffer 1 at 2 ml/min.
2. Load the sample lysate at 2 ml/min.
3. Wash the cartridge with 6 CV of wash buffer 1 at 2 ml/min.

4. Wash the cartridge with 6 CV of wash buffer 2 at 2 ml/min.
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 and gel in Figure 5 illustrate a representative purification of a high-expressing soluble protein purified using the native buffer set and method described in Table 6. 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.

Native IMAC Purification Profile

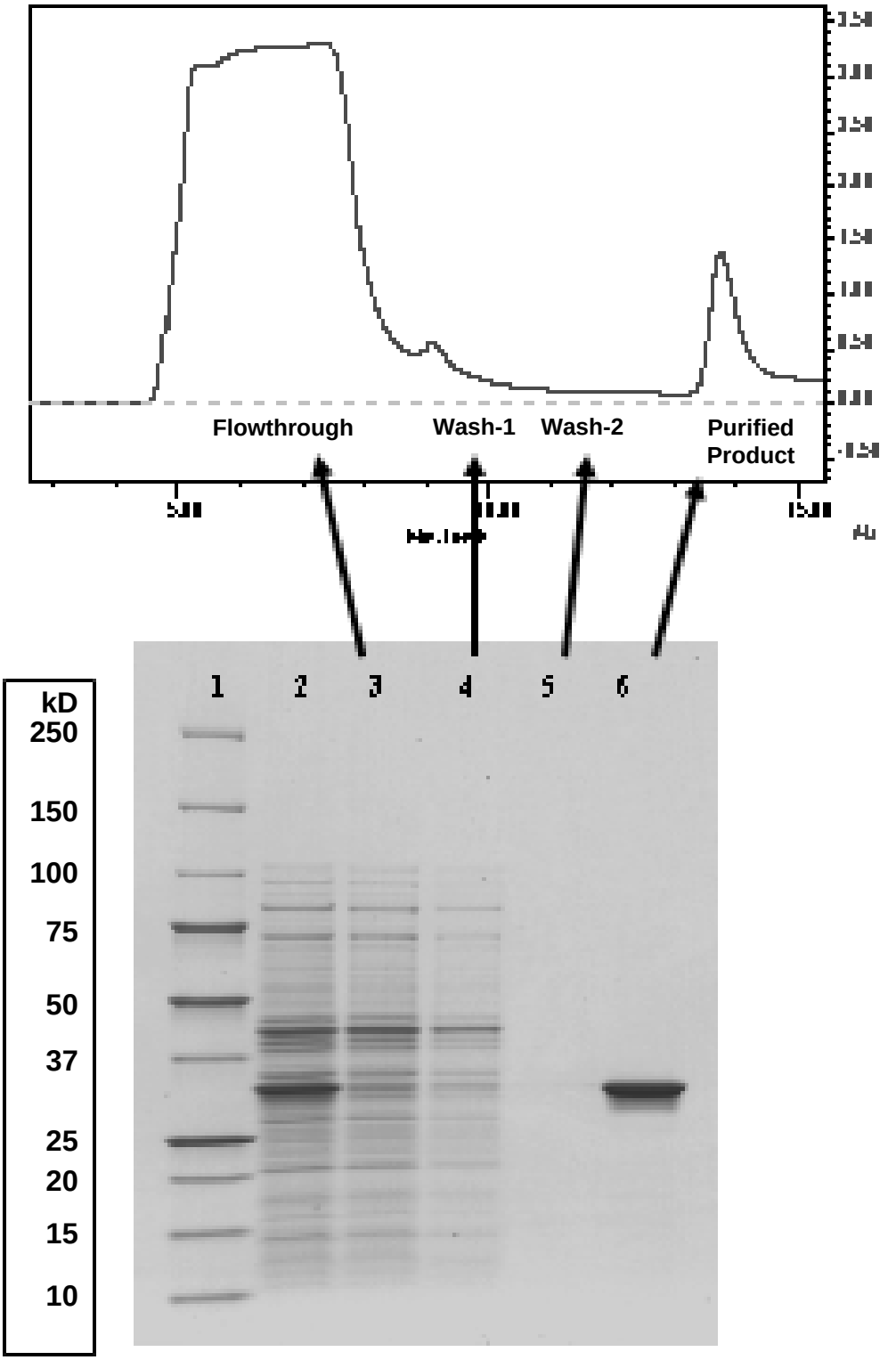


Fig. 5. Native IMAC purification: A 32 kD Nif-3* His-tagged protein was purified from the soluble fraction using the standard Profinity IMAC native purification protocol. 2 ml of lysate (2 CV) from a 100 ml *E. coli* culture was loaded onto a 1 ml IMAC cartridge. The cartridge was washed with 6 CV of wash buffer 1, followed by 6 CV of wash buffer 2, and purified protein was eluted with 5 CV of elution buffer (all at 2 ml/min). The purified product was >95% pure by densitometric scanning and Quantiy One® software analysis. Lane 1, Precision Plus Protein unstained standards; lane 2, soluble lysate; lane 3, Flow-through; lane 4, wash 1; lane 5, wash 2; lane 6, purified product.

***Nif-3 construct was kindly provided by R. Stevens, Scripps Institute.**

Section 9
Scaling Up

Bio-Scale Mini cartridges are available in 1 ml and 5 ml cartridge formats. The Profinity IMAC resin is also available in larger amounts, from 25 ml bottles to 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 care should be taken 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. Inquire with your local Bio-Rad representative or go online to www.bio-rad.com.

Section 10 Regenerating, Cleaning, Sanitizing, and Storage

Protein cross-contamination, frit clogging, and increased backpressure can result from repeating the number of uses beyond the recommended number. After repeated use, a cartridge may run slower or produce higher backpressure, an expected result due to the very nature of the sample mixture. The following cleaning and regeneration procedures may be used, however, it is recommended to dispose of the cartridge after several uses. To avoid cross-contamination, it is recommended that single cartridges are designated for single proteins.

To maintain good flow properties, it is recommended that the cartridges are cleaned between each use.

For the 1 ml cartridges, run the cleaning protocol at 2 ml/min. It is recommended that the 5 ml cartridge cleaning protocol be run 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% ETOH at 4°C.

Chaotrope 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% ETOH at 4°C.

In situations where it is desired to run different proteins over the same cartridge, a complete sanitization, stripping, and recharging is recommended between sample runs. Care should be taken with the handling and disposal 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

Problem	Possible Cause	Solution
Cartridge clogging or slow flow rate	Particulates in samples or buffers	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 eluant	Low level of target protein in starting material	Check expression level by SDS-PAGE
	Target protein not binding, or eluting in wash fractions	Check levels of target protein in lysate, flow-through, wash, and eluted fractions. Check for presence of His-tag with anti-His antibody
Target protein in flow through	His-tag not accessible	Reclone His-tag onto opposite terminus (N or C-terminus)
	His-tag not accessible	Purify protein under denaturing conditions to expose His-tag
	Proteolysis & removal of tag	Include protease inhibitors in lysis buffer (or reaction), or purify in the cold

Problem	Possible Cause	Solution
Precipitation during purification	Binding capacity of cartridge exceeded	Load less sample
	Protein aggregating	Include low levels of detergent (0.1% Triton X-100, Tween 20) in purification. Include glycerol up to 10%
	Protein too concentrated during elution	Elute with imidazole gradient (10–250 mM) rather than step elution
Eluted protein is impure	Contaminants co-eluting	Elute with imidazole gradient (10–250 mM) rather than step elution
	Contaminants co-eluting	Increase the imidazole in the wash but keep below 40 mM to increase wash stringency
Target protein is degraded	Proteolysis of target protein	Add protease inhibitors to lysate. Purify at 4°C or under denaturing conditions

Section 12
Ordering Information

Cartridges

Catalog #	Description
732-4610	Bio-Scale Mini Profinity IMAC cartridge, 5 x 1 ml cartridge
732-4612	Bio-Scale Mini Profinity IMAC cartridge, 1 x 5 ml cartridge
732-4614	Bio-Scale Mini Profinity IMAC cartridge, 5 x 5 ml cartridges

For the most up to date list of other cartridge offerings, please visit us online at www.bio-rad.com/cartridges/

Fittings, Tubing, & Fittings Kits

Catalog #	Description
731-8225	1.6 mM Barb to Male Luer
731-8222	1.6 mM Barb to Female Luer
732-0111	Luer to M6 Adaptor Fittings Kit, includes luer to M6 fitting to connect to an FPLC system
732-0112	Luer to 10-32 Adaptor Fittings Kit, includes luer to polypropylene/PTFE 10-32 fittings to connect 1 cartridge to an 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

- Larger package sizes of media are available for process scale chromatography. Inquire with your local Bio-Rad representative.

Section 13 References

Joyce AR and Palsson BO,The model organism as a system: integrating 'omics' data sets, Nat Rev Mol Cell Biol 7, 198–210 (2006)

Belew M and Porath J, Immobilized metal ion affinity chromatography. Effect of solute structure, ligand density and salt concentration on the retention of peptides, J. Chromatogr 516, 333–354 (1990)

Hochuli E, Large-scale chromatography of recombinant proteins, J Chromatogr 444, 293–302 (1988)

Maté MJ et al., The crystal structure of the mouse apoptosis-inducing factor AIF, Nat Struct Biol 9, 442–446 (2002)

Porath J et al., Metal chelate affinity chromatography, a new approach to protein fractionation, Nature 258, 598–599 (1975)

Section 14
Legal Notices

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

Purification and preparation of fusion proteins and affinity peptides comprising at least two adjacent histidine residues may require a license under US patent 5,284,933 and US patent 5,310,663, including corresponding foreign patents (assignee Hoffman-La Roche, Inc).

Bio-Rad Laboratories, Inc.
925 Alfred Nobel Dr.
Hercules, CA 94547 USA
510-741-1000
1-800-424-6723

10005455 Rev C