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

Instruction Manual

Catalog #
732-4620
732-4622
732-4624

BIO-RAD

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Section 1
Introduction

Bio-Scale Mini GST cartridges are convenient, disposable, prepacked low-pressure chromatographic cartridges. Bio-Scale Mini cartridges offer both increased run-to-run reproducibility 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 protein separation needs.

Profinity glutathione support is based on Bio-Rad's proprietary UNOsphere™ technology (US patent 6,423,666) for capture and purification of glutathione S-transferase (GST)-tagged proteins. Its ligand density has been optimized for maximum capture of target proteins. Ideal for scale-up, the Profinity glutathione support's open pore structure is ideal for purifying proteins of a wide molecular weight range. (Smith DB et al., 1988)

Bio-Scale Mini GST cartridges are packed with

Bio-Rad's innovative Profinity GST resin. Structural characteristics such as the polymeric nature, optimized ligand density, and open pore structure of the Profinity GST bead result in superb mechanical strength and performance of the prepacked cartridges.

Section 2
Product Information

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), and affinity (AC) chromatography. See Ordering Information for a listing of the complete Bio-Scale Mini cartridge product line.

Bio-Scale Mini cartridges are quickly connected to liquid chromatography systems or luer-end 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 GST 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: 1.0–2.0 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 or 2% Benzyl Alcohol
Autoclavability	Not autoclavable

Table 2. Profinity GST Resin Specifications

Functional ligand	Glutathione Derivative
Base bead	UNOsphere
Particle size range	45–90 µm
Mean particle size	70 µm
Functional group density	≥60 µmol/g
Dynamic binding capacity*	>11 mg/ml
Recommended linear flow rate	<600 cm/hr at 25°C
Maximum operating pressure	≥43 psi
Chemical compatibility	See table 3
Storage	4–8°C
Shelf life in 20% EtOH	>1 year at 4–8°C
Operational temperature	4–40°C

* 60% Breakthrough for a purified 51 kD GST fusion protein at 0.5 ml/min. Note: Dynamic binding capacity is a function of a number of factors including pH, flow rate, and sample temperature.

Profinity GST cartridges are compatible with aqueous buffers most commonly used with GST purification techniques.

Table 3. Buffer and Chemical Compatibilities for Profinity GST cartridges

Reagent	Stability
Buffer reagents	
Tris	50 mM
HEPES	50 mM
MOPS	50 mM
Sodium or potassium phosphate	50 mM
Chelating agents	
EDTA, EGTA	5 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%
Zwitterionic detergents (CHAPS, CHAPSO)	5%
Anionic detergents (SDS, Sarkosyl)	1%
Denaturing agents (for cleaning only)	
Guanidine-HCl	6 M
Urea	8 M
Other additives	
NaCl	2 M
$MgCl_2$	100 mM
$CaCl_2$	10 mM
Glycerol	20%
Ethanol	20%
Citrate	80 mM

Section 3
Connection to Low-Pressure Chromatography Systems

Bio-Scale Mini cartridges are ideal for use with Bio-Rad's BioLogic™ LP chromatography system, Econo™ Gradient pump, the patented* Model EP-1 Econo pump, and all low-pressure chromatography instruments. Bio-Scale Mini cartridges can be conveniently connected directly to the system using the luer fittings on the cartridge.

1. Install 1.6 mm inner diameter (ID) tubing in the pumphead. Adjust the platen pressure screw (on pumphead) using a screwdriver or coin. Turn the screw counterclockwise as far as it will go, then turn clockwise three full turns. Assemble with fittings and lock rings as shown in Figure 1.

* US patent 5,135,658

(Use orange lock rings and medium size barb fittings with 1.6 mm tubing.)

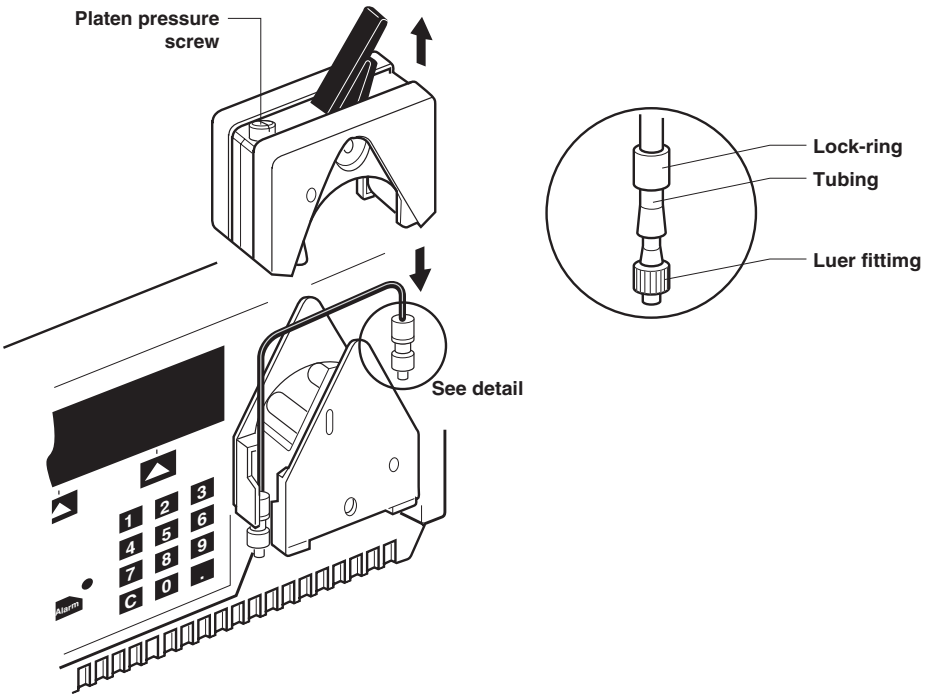

Fig. 1. BioLogic LP system setup.

2. To maximize gradient accuracy and to apply samples efficiently, install 1.6 mm ID tubing from the pump to the MV-6 sample inject valve (if available). If using the MV-6 sample inject valve, turn the knob counterclockwise as far as it will go so it will now correspond to the printed diagram on the valve (see Figure 2).

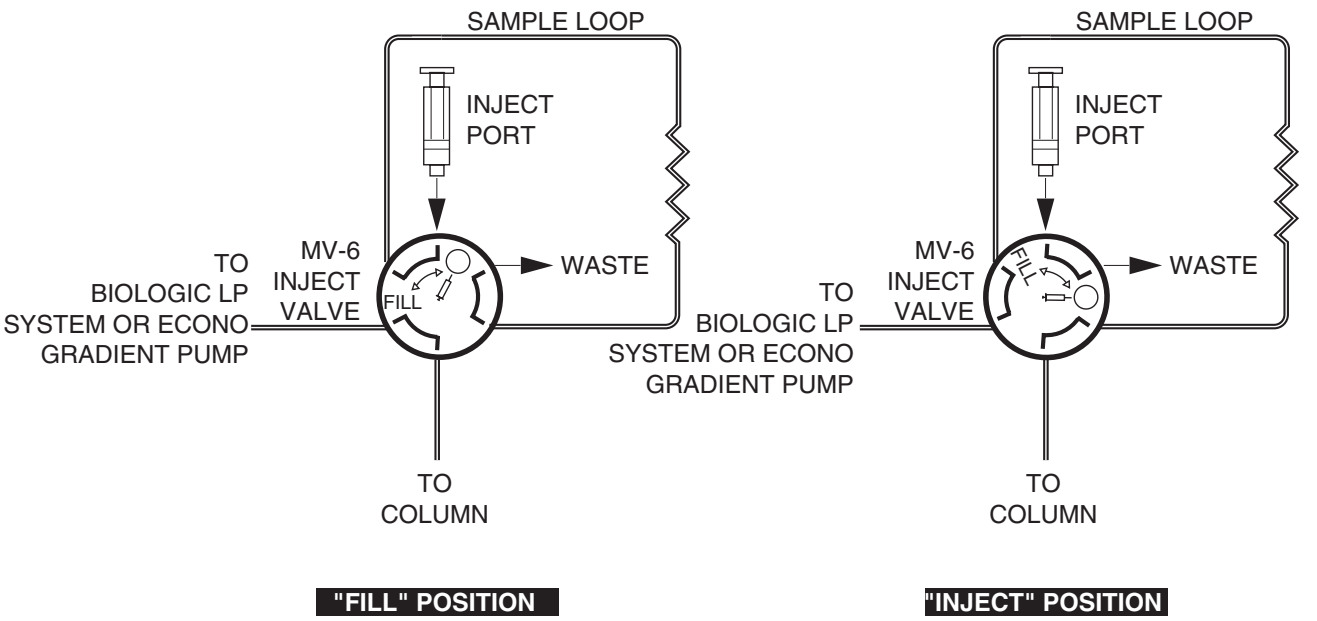

Fig. 2. Connecting to an MV-6 valve.

3. Connect the inlet of the cartridge to the male luer fitting on the MV-6 sample inject valve (see Figure 2). If not using the MV-6 sample inject valve, connect a barb to male luer fitting on the 1.6 mm ID tubing, then connect to the top of the female luer on the Bio-Scale Mini cartridge. For optimum performance, a cartridge should be mounted vertically with the arrow on the cartridge pointing downward (see Figure 3).

4. Connect the cartridge outlet to the 1.6 mm ID tubing leading to the BioLogic LP system optics module or to the Model EM-1 Econo UV monitor. It is recommended to use the shortest length (approximately 10 cm) of 1.6 mm ID tubing. Connect a barb to female luer to the 1.6 mm ID tubing, then connect to the bottom of the male luer on the Bio-Scale Mini cartridge.

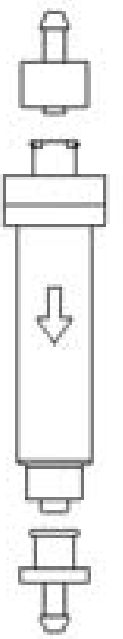

Fig. 3. Cartridge and fittings. Luer fittings and column: a cartridge should be mounted vertically with the arrow on the cartridge pointing downward.

Section 4
Connection to Medium and High-Pressure Chromatography Systems

Bio-Scale Mini cartridges can be connected to any liquid chromatography system, provided that the maximum pressure limit (3 bar, 45 psi, or 300 kPa) of the cartridges is not exceeded. It is recommended that the system pressure limit be set according to the cartridge pressure limit. Pressures in excess of 3 bar are usually caused by restrictions in tubing or detector cells downstream from the cartridge.
Bio-Rad offers two fitting kits for easy connection of a Bio-Scale Mini cartridge to a BioLogic DuoFlow, HPLC, or FPLC-type system.

4.1 BioLogic DuoFlow Systems

The luer to BioLogic system fittings kit (catalog #732-0113) includes 1/4-28 female to male luer and 1/4-28 female to female luer to connect one Bio-Scale Mini cartridge to the BioLogic DuoFlow system. (see Figure 4)

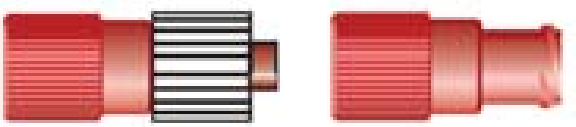

Fig. 4. Luer to 1/4-28 adaptor.

4.2 HPLC Systems

The luer to 10-32 adaptor fittings kit (catalog #732-0112) provides fittings necessary to connect the Bio-Scale Mini cartridge to nut and ferrule type fittings found on most HPLC systems. Alternatively, the cartridge can be connected to HPLC systems via a low dead-volume 1/16 inch union with a new piece of stainless-steel tubing attached to the union. Simply slip a short length of the 0.8 mm ID tubing over the 1/16 inch OD stainless-steel tubing to a distance of 1 cm.

4.3 FPLC Systems

The luer to M6 adaptor fittings kit (catalog #732-0111) provides fittings necessary to connect the Bio-Scale Mini cartridge to the M6 fittings found on FPLC or related systems. Alternatively, connection can be made by using one GE Healthcare Union Luerlock female to M6 female fitting (GE 18-1027-12) and

one Upchurch P-686, female slip luer to male M6 fitting or GE 18-1027-62, Union luerlock female to M6 male fitting. To prevent tubing or cartridge failure, do not exceed the maximum recommended flow rate of the cartridge.

* Fittings kit ordering information can be found within the Ordering Information section of this manual.

Section 5
Buffers and Methods

GST methods can be run using only native purification protocols. Under native conditions, proteins are purified using buffers that help retain the natural folded structure of the target protein. The recommended buffer compositions are provided in Table 4.

Table 4. Recommended Buffers and Storage Solutions

Solution	Composition
Lysis/wash buffer	150 mM NaCl, 10 mM Na_2HP0_4, 5 mM EDTA, pH 7.4
Elution buffer	20 mM glutathione, 100 mM Tris, 5 mM EDTA, pH 8.0
Desalting buffer	137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HP0_4, 8.1 mM KH_2P0_4, pH 7.4
Cleaning solution 1	500 mM NaCl, 50 mM Tris, pH 8.0
Cleaning solution 2	500 mM NaCl, 100 mM NaOAc, pH 4.5
Storage solution	2% Benzyl alcohol or 20% Ethanol

Section 6
Quick Solubility Screening Protocols

Before choosing a 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 purified with the native purification 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 5.

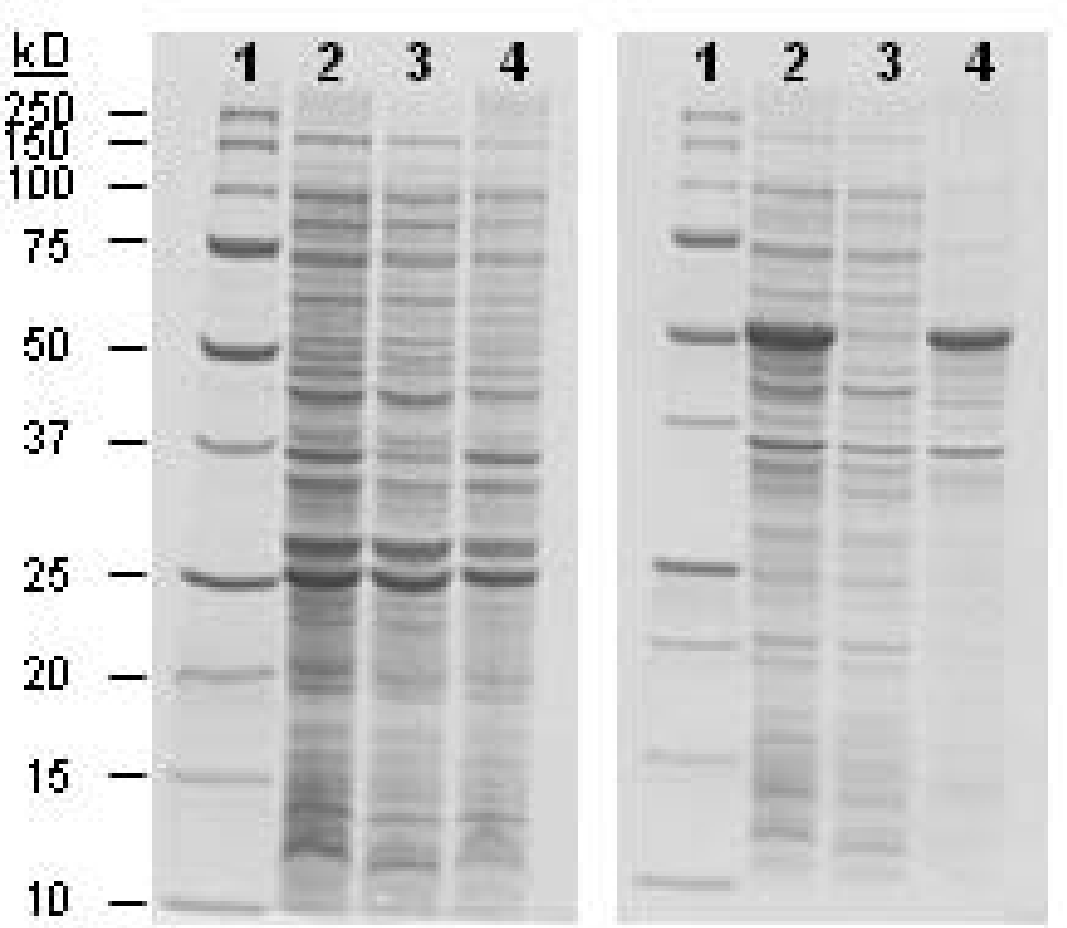

Fig. 5. Partioning profiles. Representative gels showing partitioning of the target protein into the soluble fraction (left panel) or insoluble fraction (right panel). For both gels, Precision Plus Protein™ standards were loaded in lane 1, followed by the total, soluble, and insoluble fractions in lanes 2–4 respectively. The first panel depicts GST, a 26 kD protein, which partitions into the soluble fraction. The second panel shows GST-tagged GFP, grown under conditions that drive the fusion protein into inclusion bodies.

Section 7
Preparation of *E. coli* Lysates

Lysates from *E. coli* cultures can be prepared using conventional sonication procedures with the lysis buffers supplied in each kit, or can be prepared using chemical lysis methods and the Profinia™ bacterial lysis/extraction reagent. For *E. coli* cultures expressing medium to high levels of fusion proteins, (≥10% of total protein), 200 ml of culture will normally 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. Bacterial cultures can be grown in advance and centrifuged. The pellets can be stored at –70°C for several months and lysed at a convenient date for sample preparation.

Basic Protocol

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 of lysis/wash buffer (200 ml of culture typically yields 0.8–1.0 g of paste, or 8–10 ml of lysate).
3. Thoroughly resuspend the pellet by pipetting or vortexing.
4. As an optional step and to decrease the viscosity, add a nuclease solution (DNase at 100 units/ml or Benzonase at 25 units/ml) and incubate for 10 min at room temperature).
5. Sonicate the lysate (on ice, using 25% output) 4 times at 1 min intervals.
6. Centrifuge the lysate at 16,000 x g for 20 min at 4°C.
7. Remove the supernatant and filter through a 0.45 µm filter to remove particulates. The lysate is now ready to be loaded into the Bio-Scale Mini Profinity GST cartridge.

If the lysate is not going to be used immediately, it can be frozen at –20°C and thawed once to be purified at a later date. However, proteolysis can occur upon freezing and thawing, and the quality of the purified product may be compromised. This will have to be determined empirically for individual proteins. Upon thawing, refilter through a 0.45 µm filter, as precipitates often form after freezing.

Section 8
Preparing a Cartridge, and Subsequent Purification

Prepare buffer sets for the 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

or buffer of choice, 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 5. 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	0.5–1 ml/min	2.5–5 ml/min
Wash 1	12	2 ml/min	10 ml/min
Elute	3	0.5 ml/min	2.5 ml/min

Standard methods that are compatible with any type of chromatography system are listed below. To maximize binding capacity, the lysate load flow rate can be decreased to the minimum recommended flow rate for 1 ml and 5 ml columns (Table 1). This 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 0.5–1 ml/min.
3. Wash the cartridge with 12 CV of wash buffer at 2 ml/min.
4. Elute the purified protein with 3 CV of elution buffer at 0.5 ml/min.
5. Collect the fractions of eluted target protein for analysis by SDS-PAGE and pool the fractions that are satisfactory.

Cleaning the Cartridge

6. Wash the column with 5 CV of cleaning buffer 1 at 2 ml/min.
7. Wash the column with 5 CV of cleaning buffer 2 at 2 ml/min. Rinse the column with 5 CV of high-purity deionized water at 2 ml/min.
8. Rinse the column with 5 CV of storage solution at 1 ml/min.
9. Store the cleaned column well-sealed at 4ºC.

The chromatogram and gel in Figure 6 illustrate a representative purification of a high-expressing soluble protein purified using the GST buffer set and method described in Tables 4 and 5.

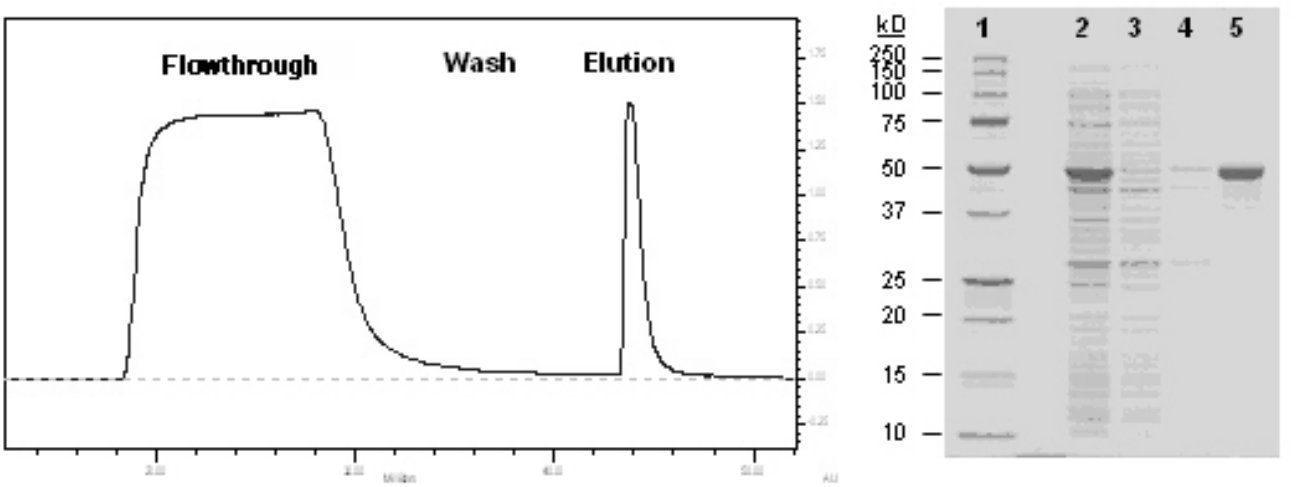

Fig. 6. GST purification: A 51 kD GST-tagged protein was purified from the soluble fraction of an *E. coli* lysate using a standard Profinity GST purification protocol. 10 ml of lysate (10 CV) from a 100 ml *E. coli* culture was loaded onto a 1 ml Profinity GST cartridge. The cartridge was washed with 12 CV of wash buffer and purified protein was eluted with 3 CV of elution buffer (0.5 ml/min). The purified product was >80% pure by densitometric scanning and Quantiy One® software analysis. Lane 1, Precision Plus Protein unstained standards; lane 2, soluble lysate; lane 3, flowthrough; lane 4, wash 1; lane 5, purified product.

Cleavage of GST fusion proteins

Design of an enzyme-cleavable fusion construct requires splicing a recognition site for thrombin, Factor Xa, or other sequence-specific proteolytic enzyme into the linkage between the GST and the target protein. The target protein can be obtained in purified form post-elution, or while still on the column (Dian C et al. 2002).

Section 9
Scaling Up

Bio-Scale Mini cartridges are available in 1 ml and 5 ml cartridge formats. The Profinity GST 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 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, single cartridges should be designated for single proteins.

To maintain good flow properties, the cartridges should be cleaned between each use. For the 1 ml cartridges, run the cleaning protocol at 3 ml/min. It is recommended that the 5 ml cartridge cleaning protocol be run at 15 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 or 2% Benzyl alcohol at 4–8°C.

Chaotropic Agent 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 or 2% Benzyl alchol at 4–8°C.

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 eluate	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, flowthrough, wash, and eluted fractions. Check for presence of tag with anti-GST antibody
Target protein in flowthrough	Tag not accessible	Reclone GST-tagged protein onto opposite terminus (N- or C-terminus)
	Tag not accessible	Purify protein under denaturing conditions to expose glutathione S-tranferase tag
	Proteolysis and 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	Eluate with elution buffer gradient
Eluted protein is impure	Contaminants coeluting	Decrease sonication time
Target protein is degraded	Proteolysis of target protein	Add protease inhibitors to lysate. Purify at 4°C or under denaturing conditions
Low yield	Low expression level	Optimize expression system or use different system
	Insufficient extraction	Use frozen bacterial pellet instead of fresh pellet. Adding lysozyme to the lysis buffer may increase the efficiency of extraction
	Construct does not bind to column	Fusion partner affects GST conformation. Adding in 5 mM DTT to lysis buffer may help
Low product purity	Construct binds other bacterial	Adding DTT can reduce nonspecific interactions

Problem	Possible Cause	Solution
		A small amount of a nondenaturing detergent can be added to the wash buffer
	Column not washed sufficiently	Increase number of column volumes in wash buffer step
Column runs slowly	Overloading column	Reduce lysate load volume
	Sample is too viscous	Dilute the lysate before application to column

Section 12
Ordering Information

Bio-Scale Mini Cartridges

Description	5 x 1 ml	1 x 5 ml	5 x 5 ml
UNOsphere Q Support	732-4100	731-4102	731-4104
UNOsphere S Support	732-4110	731-4112	731-4114
Macro-Prep® High Q Support	732-4120	732-4122	732-4124
Macro-Prep High S Support	732-4130	732-4132	732-4134
Macro-Prep DEAE Support	732-4140	732-4142	732-4144
Bio-Gel P-6 Support	—	732-4502	732-4504
Affi-Prep® Protein A Support	732-4600	732-4602	—
Profinity IMAC Support	732-4610	732-4612	732-4614
Profinity GST Support	732-4620	732-4622	732-4624
Affi-Gel® DEAE Blue Support	–	732-4642	732-4644
Affi-Gel Blue Support	–	732-4632	732-4634

		1x	5x
Bio-Gel P-6 Desalting, 10 ml	—	—	732-5304
Bio-Gel P-6 Desalting, 50 ml	—	732-5312	732-5314

- Visit **www.bio-rad.com/cartridges/** for current information on prepacked cartridges.
- Larger package sizes of media are available for process-scale chromatography. Inquire with your local Bio-Rad representative.

Fittings Kits

Catalog #	Description
732-0111	Luer to M6 Adaptor Fittings Kit, includes luer to M6 fittings to connect 1 cartridge to an FPLC system
732-0112	Luer to 10-32 Adaptor Fittings Kit, includes luer to 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 a BioLogic DuoFlow system

Section 13
References

Dian C et al., Strategies for the purification and on-column cleavage of glutathione S-transferase fusion target proteins, J Chromatogr B Analyt Technol Biomed Life Sci 769, 133–144 (2002)

Smith DB and Johnson KS, Single-step purification of polypeptides expressed *Escherichia coli* as fusions with glutathione S-transferase, Gene 67, 31–40 (1988)

Section 14
Legal Notices

Benzonase is a trademark of Novage. FPLC is a trademark of GE Healthcare. Luer-Lok is a trademark of Becton, Dickenson & Co. 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).

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