EconoFit Profinity GST Columns, 1 and 5 ml

Instruction Manual

Catalog #12009295
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12009297

Please read the instructions in this manual prior to using EconoFit Profinity GST Columns. If you have any questions or require any further assistance, please contact your Bio-Rad Laboratories representative.

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Section 1
Introduction

EconoFit Profinity GST Columns are convenient, disposable, prepacked low-pressure chromatography columns. They facilitate both increased run-to-run uniformity and high purity of proteins through the column design and novel resin technology. Compatible with most aqueous buffers commonly used for protein purification, EconoFit Columns offer improved performance for your protein separation needs.

Profinity glutathione support is based on Bio-Rad's proprietary UNOsphere technology (U.S. 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 et al. 1988).

EconoFit Profinity GST Columns are packed with Bio-Rad's innovative Profinity GST Resin. Structural characteristics of the Profinity GST bead, such as the polymeric nature, optimized ligand density, and open pore structure, result in superb mechanical strength and performance of the prepacked columns.

Section 2
Product Information

EconoFit Columns are disposable, easy-to-use, prepacked chromatograhic columns, which are supplied ready for use in convenient 1 and 5 ml sizes. They can be quickly connected to liquid chromatography systems using 10-32 fittings. Columns are available for a variety of chromatographic techniques, including desalting (size exclusion), ion exchange, affinity, mixed-mode, and hydrophobic interaction chromatography. See Tables 1 and 2 for column and resin specifications, respectively. Refer to **bio-rad.com/ResinsandColumns** for a complete listing of products in the EconoFit Column portfolio.

Table 1. EconoFit Profinity GST Column specifications.

Property	Description
Size	1 ml and 5 ml bed volumes
Bed dimensions	1 ml: 25 mm length x 7 mm inner diameter 5 ml: 25 mm length x 16 mm inner diameter
Maximum pressure tolerance	72 psi
Recommended flow rate	1 ml: 1–2 ml/min (240–480 cm/hr) 5 ml: 5–10 ml/min (240–480 cm/hr)
Fittings	10-32 (1/16"), female inlet and male outlet
Column material	Polypropylene
Frit material	High-density polyethylene
Shipping solution	20% ethanol
Storage conditions	20% ethanol or 2% benzyl alcohol
Autoclavability	Not autoclavable
Shelf life	3 years at 4°C

Table 2. Profinity GST Resin specifications.

Property	Description
Functional ligand	Glutathione derivative
Base bead	UNOsphere
Particle size	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% ethanol	>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 Columns are compatible with most aqueous buffers commonly used with GST purification techniques (see Table 3).

Table 3. Buffer and chemical compatibilities for Profinity GST Columns.

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	
ß-mercaptoethanol	30 mM
Dithiothreitol	5 mM
TCEP	10 mM
Detergents	
Nonionic detergents (Triton X-100, Tween 20, 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
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 4
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 *Escherichia coli* culture by centrifugation at 4,000 x g for 10 min at 4°C.
2. Resuspend the pellet in 500 µl of phosphate buffered saline (PBS) and sonicate for 60 sec, on ice, in 10 sec pulses. Remove 50 µl of the sonicate and label it 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 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 it 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 1.

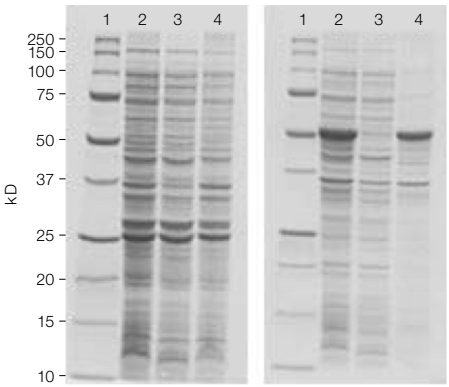

Fig. 1. Partitioning profiles. Representative gels show partitioning of the target protein into the soluble fraction (left panel) or insoluble fraction (right panel). For both gels, Precision Plus Protein Standards were loaded in lane 1, followed by the total, soluble, and insoluble fractions in lanes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

Section 5
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 they 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 column purification, and 1,000 ml of culture will yield sufficient material for a 5 ml column purification. 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 ten 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) four 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 EconoFit Profinity GST Column.

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 6
Preparing a Column and Subsequent Purification

Prepare buffer sets for the purification protocols using a single buffer set throughout the procedure. To prepare the column for the procedure, remove the top closure and connect the column to the chromatography system. Open the bottom closure and connect the column outlet to the system.

Flush the packing solution (20% ethanol) from the column by running 2 column volumes (CV) of water or buffer of choice at a flow rate of 2 ml/min (1 ml column) or 10 ml/min (5 ml column). The column is now ready for the purification steps. Flow rates are given in ml/min and are specific to the 1 ml column. If a 5 ml column is used for a procedure, substitute the higher flow rate in the method (see Table 5).

Table 5. Purification method suggestions.

Step	CV	1 ml Column Flow Rate	5 ml Column Flow Rate
Equilibrate	5	2 ml/min	10 ml/min
Load lysate	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

Follow the instructions in Standard Method, which is compatible with any type of chromatography system. 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.

Standard Method

1. Equilibrate the column 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 column 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 Column

1. Wash the column with 5 CV of cleaning buffer 1 at 2 ml/min.
2. 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.
3. Rinse the column with 5 CV of storage solution at 1 ml/min.
4. Seal the cleaned column well and store it at 4ºC.

The chromatogram and gel in Figure 2 illustrate a representative purification of a high-expressing soluble protein purified using the GST buffer set and method described in Tables 4 and 5.

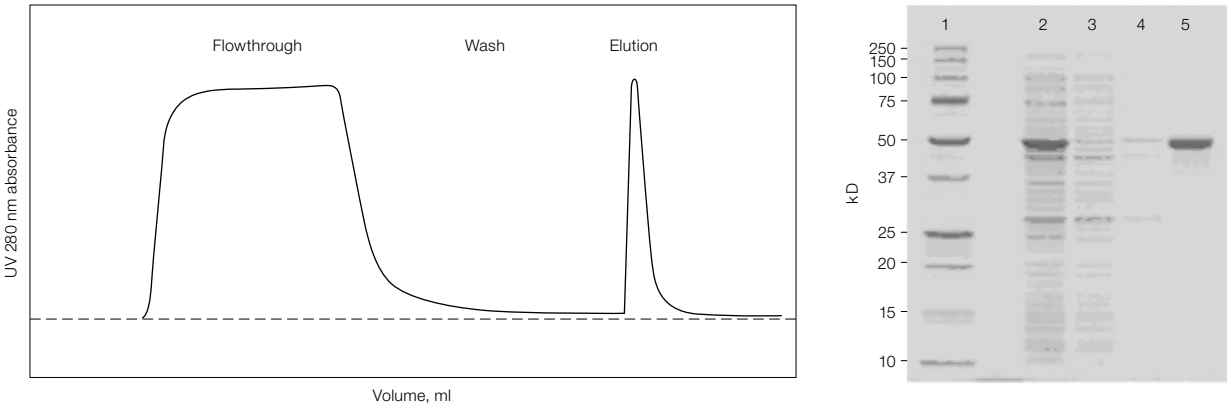

Fig. 2. GST purification. A 51 kD GST-tagged protein was purified from the soluble fraction of an *E. coli* lysate using a standard Profinity GST purification protocol. 10 ml of lysate (10 CV) from a 100 ml *E. coli* culture was loaded onto a 1 ml Profinity GST Column. The column was washed with 12 CV 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 Quantity 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 postelution or while still on the column (Dian et al. 2002).

Section 7
Scaling Up

EconoFit Profinity GST Columns are available in 1 and 5 ml sizes.

For quick scale-up, two or three columns of the same type can be connected in series. Backpressure will increase with columns in series, so care should be taken to maintain an overall system pressure ≤72 psi.

In addition, Bio-Rad carries an extensive line of empty chromatography columns from laboratory to process scale. Go to **bio-rad.com/ResinsandColumns** or inquire with your local Bio-Rad representative.

Section 8
Regenerating, Cleaning, Sanitizing, and Storage

Protein cross-contamination, frit clogging, and increased backpressure can result from running a column beyond the recommended number of uses. After repeated use, a column may run slower or produce high backpressure. We recommend that you dispose of a column after several uses. To avoid cross-contamination, designate each column for a single protein. To maintain good flow properties, clean the columns between uses.

High Salt/Acid Cleaning

1. Rinse the column with 2 CV water at 2 ml/min.
2. Wash the column with 5 CV 500 mM NaCl, 50 mM Tris, pH 8.0, at 2 ml/min.
3. Wash the column with 5 CV 500 mM NaCl, 100 mM NaOAc, pH 4.5, at 2 ml/min.
4. Rinse the column with 2 CV water at 2 ml/min.
5. Store the column in 20% ethanol or 2% benzyl alcohol at 4–8°C.

Chaotropic Agent Cleaning

1. Rinse the column with 2 CV water at 2 ml/min.
2. Wash the column with 5 CV 6 M guanidine HCl at 2 ml/min.
3. Rinse the column with 2 CV water at 2 ml/min.
4. Store the column in 20% ethanol or 2% benzyl alcohol at 4–8°C.

Section 9
Troubleshooting Guide

Problem	Possible Cause	Solution
Column 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) ▪ Purify protein under denaturing conditions to expose GST tag
	Proteolysis and removal of tag	Include protease inhibitors in lysis buffer (or reaction), or purify in the cold
Precipitation during purification	Binding capacity of column 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 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 ▪ Add lysozyme to the lysis buffer, which may increase the efficiency of extraction
	Construct does not bind to column	Fusion partner affects GST conformation. Adding 5 mM dithiothreitol to lysis buffer may help
Low product purity	Construct binds other bacterial proteins	▪ Adding dithiothreitol can reduce nonspecific interactions ▪ 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 10
Ordering Information

Catalog #	Description
12009295	**EconoFit Profinity GST Columns**, 5 x 1 ml
12009296	**EconoFit Profinity GST Column**, 1 x 5 ml
12009297	**EconoFit Profinity GST Columns**, 5 x 5 ml

Larger package sizes of media are available for process-scale chromatography.
Go to **bio-rad.com/ResinsandColumns** or inquire with your local Bio-Rad representative.

Section 11
References

Dian C et al. (2002). 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.

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

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