EconoFit Profinity eXact Columns, 1 and 5 ml

Instruction Manual

Catalog number

12009292
12009293
12009294

Please read the instructions in this manual prior to using EconoFit Profinity eXact 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 eXact Columns are convenient, disposable, prepacked low-pressure chromatography columns. EconoFit Columns offer both increased run-to-run uniformity and high purity of protein through the column design and novel resin technology. Compatible with aqueous buffers most commonly used for protein purification, EconoFit Columns offer improved performance for your protein separation needs.

EconoFit Profinity eXact Columns are packed with Profinity eXact (exact affinity cleavage technology) Purification Resin. This agarose-based affinity chromatography resin utilizes an immobilized, modified protease that selectively binds the fusion protein and cleaves the affinity tag on-column under controlled conditions, releasing the purified target protein containing only its native amino acid sequence. This innovative resin technology improves the efficiency of recombinant protein purification and is the only affinity chromatography platform that completes the purification and tag removal process in a single step.

Section 2
Product Information

EconoFit Columns are disposable, easy-to-use, prepacked chromatographic columns supplied ready for use in convenient 1 and 5 ml sizes. They are quickly connected to liquid chromatography systems using 10-32 fittings. Columns are available for a variety of chromatographic techniques, including desalting (size exclusion [SEC]), ion exchange (IEX), affinity (AC), mixed-mode, and hydrophobic interaction chromatography (HIC). See Table 1 for specifications. Refer to **bio-rad.com/ResinsandColumns** for a complete listing of items in the EconoFit Column product line.

Table 1. EconoFit Profinity eXact Column specifications.

Property	Description
Size	1 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
Maximum flow rate	1 ml: 3 ml/min (730 cm/hr) 5 ml: 15 ml/min (722 cm/hr)
Fittings	10–32 (1/16"), female inlet and male outlet
Column material	Polypropylene
Frit material	High-density polyethylene
Shipping solution	100 mM sodium phosphate, 0.02% sodium azide, pH 7.2
Storage conditions	100 mM sodium phosphate, 0.02% sodium azide, pH 7.2
Autoclavability	Not autoclavable

Profinity eXact Resin is also available in bottles. Refer to Ordering Information in section 10 of this manual. See Table 2 for specifications. Go to **bio-rad.com/ResinsandColumns** for more information.

Table 2. Profinity eXact Resin specifications.

Functional ligand	Mutant subtilisin
Base bead	Superflow 6% agarose
Particle size range	60–160 µm
Recommended linear flow rate	<1,000 cm/hr at 25°C
Maximum operating pressure	72 psi
Chemical compatibility	See Table 3
Storage	4°C
Shelf life in storage buffer	>1 year at 4°C
Operational temperature	4–40°C

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

Reagent Group	Description	Stability
Triggering ions	F^-, Cl^-, N_3^-, NO_2^-, CO_2^-	Do not use in lysis and wash buffers. See Table 5 for use as trigger in elution buffer
Salts	Sodium acetate NaCl or KCl	Do not use in lysis and wash buffers
Buffers	Tris-HCl	Substitute Tris-acetate or Tris-phosphate
Acids	HCl	Do not use. Substitute acetic or phosphoric acid
Detergents	Non-ionic Zwitterionic Ionic	≤5% (w/v) ≤5% (w/v) Do not use
Protease inhibitors	PMSF, Calbiochem cocktail, Roche cOmplete Mini Protease Inhibitor Tablet	1x
	BD Biosciences cocktail	2x
Lysis solutions	Bio-Rad ReadyPrep Protein Extraction Kit and MicroRotor for Cell Lysis Kits, B-PER Reagent (Thermo Fisher Scientific), Bug-Buster Protein Extraction Reagent (EMD Millipore), FastBreak Cell Lysis Reagent (Promega)	1x
	CelLytic Express (Sigma-Aldrich)	Do not use
Denaturants	Guanidine-HCl	Do not use in lysis and wash buffers
	Urea	Up to 2 M with no drop in binding capacity. At 4 M there may be some loss of binding capacity. At 8 M, binding capacity and target protein purity will be reduced
Other additives	$CaCl_2$	≤5 mM when used with MES, MOPS, or PIPES buffers. Do not use with phosphate buffers
	$MgCl_2$	≤5 mM. Do not use with fluoride-containing buffers. Use NaN_3 as an alternate triggering ion

For a complete list of chemical compatibilities, refer to the Profinity eXact Protein Purification System Instruction Manual (#10011260).

Section 3
Buffers and Methods

EconoFit Profinity eXact Columns 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, a strong chaotrope (such as urea) is included in the bind/wash buffer and elution buffer, allowing target proteins to be purified in an unfolded or partially folded state. The recommended buffer compositions and triggering anions are provided in Tables 4 and 5. Note that some loss in binding capacity may be observed when buffers used contain greater than 2 M urea. Additionally, urea concentrations greater than 4 M may result in decreased target protein purity.

Table 4. Buffer composition.

Bind/wash buffer*	100 mM sodium phosphate, pH 7.2
Elution buffer*	100 mM sodium phosphate, 100 mM sodium fluoride, pH 7.2
Regeneration	100 mM phosphoric acid
Storage	0.02% sodium azide, 100 mM sodium phosphate, pH 7.2

* Add urea to the bind/wash buffer and elution buffer in order to purify proteins under denaturing conditions.

Table 5. Triggering anions.

Anion	Compound	Fast Cleavage	Slow Cleavage
F^-	NaF, KF	100 mM	5 mM
N_3^-	NaN_3	10 mM	1 mM
NO_2^-	$NaNO_2$	5 mM	1 mM
CO_2^-	$NaHCO_2$	1,000 mM	25 mM
Cl^-	NaCl, KCl	>1,000 mM	75 mM

Section 4
Quick Solubility Screening Protocol

Before choosing a native or denaturing purification protocol, it is useful to determine the approximate expression level of a protein and to determine 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 can be solubilized in urea and 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 16,000 x g for 1 min at 4°C.
2. Resuspend the pellet in 500 µl of bind/wash buffer 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 16,000 x g for 5 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 bind/wash buffer containing 4 M urea. Centrifuge the lysate at 16,000 x g for 5 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 heat 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 a soluble target protein can be seen in Figure 1.

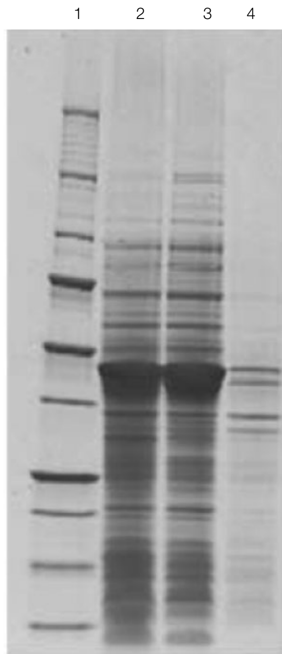

Fig. 1. Partioning profile. Precision Plus Protein Standards were used as the molecular weight marker and were loaded in lane 1, followed by the total, soluble, and insoluble fractions in lanes 2–4, respectively. The gel depicts maltose-binding protein tagged with Profinity eXact, which partitions into the soluble fraction and can be purified using the native purification protocol. A representative chromatogram and gel for the purification of this target protein is shown in Figure 2.

Section 5
Preparation of *E. coli* Lysate

For *E. coli* cultures expressing medium to high levels of proteins tagged with Profinity eXact (≥10% of total protein), 50 ml of culture will yield sufficient material for a 1 ml column purification run, and 250 ml of culture will yield sufficient material for a 5 ml column 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.

Lysate Preparation

1. Harvest cells by centrifugation.
2. Determine weight of cell pellet and resuspend in 10 volumes of bind/wash buffer (50 ml of culture typically yields 0.5 g of paste, resulting in approximately 5 ml of lysate).
3. Sonicate the lysate (on ice) for 3 min.
4. Centrifuge the lysate at ≥16,000 x g for 30 min at 4°C.
5. Remove the supernatant and filter through a 0.45 µm filter before applying to the column.

Section 6
Preparing a Column and Subsequent Purification

To prepare a column, remove the end plugs and connect the column to the chromatography system. The column is now ready for use. The following 1 ml column purification protocol is a general guideline for first time users. Flow rates for 5 ml column are shown in parentheses. The kinetics of cleavage are target protein specific and may require optimization to maximize yield. See Figure 2 for a representative purification profile using the following protocol. Refer to the Profinity eXact Protein Purification System Manual (#10011260) for complete details.

Purification Protocol

1. Equilibrate the column with 10 column volumes (CV) of bind/wash buffer at 3 ml/min (10 ml/min).
2. Load the sample lysate at 1 ml/min (5 ml/min).
3. Wash the column with 10 CV of bind/wash buffer at 3 ml/min (10 ml/min).
4. Equilibrate column with 2 CV of elution buffer at 3 ml/min (10 ml/min).*
5. Stop flow and incubate for 30 minutes.**
6. Elute purified protein with 5 CV of elution buffer at 3 ml/min (10 ml/min).

* Alternative elution protocol: Pump 5 CV of elution buffer at 0.1 ml/min (0.5 ml/min) at room temperature.
** Increased incubation time, 2–4 hr at 25°C or 12–24 hr at 4°C, may be necessary for complete cleavage of some proteins.

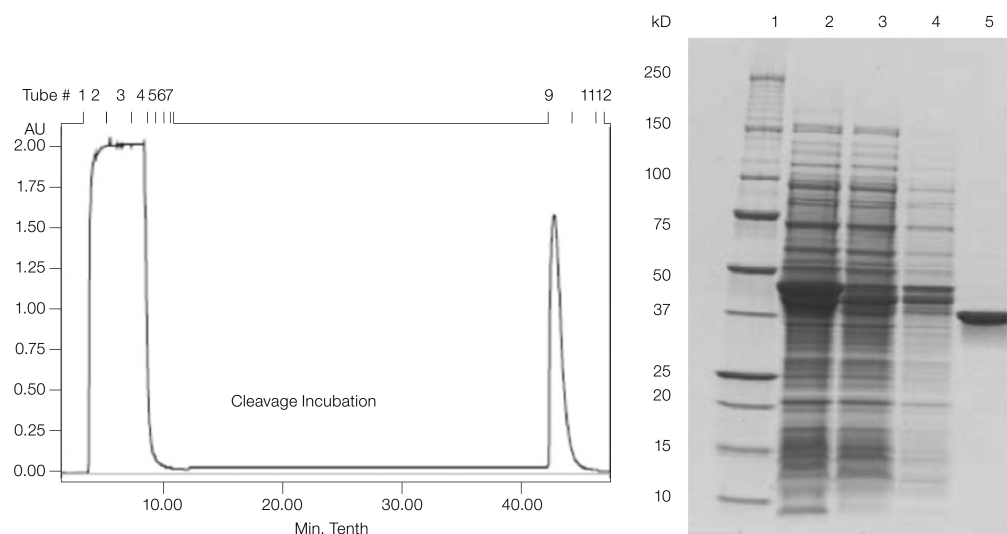

Fig. 2. Native purification. Purification of maltose-binding protein tagged with Profinity eXact from the soluble fraction using a BioLogic DuoFlow Chromatography System. Lysate (5 ml; 5 CV) prepared from 50 ml of *E. coli* culture was loaded onto a 1 ml column. The column was washed with 10 CV of bind/wash buffer, and purified protein was eluted with 5 CV of elution buffer following a 30 min cleavage incubation. The purified product was determined to be 99.5% pure by Quantity One Software analysis. Lane 1, Precision Plus Protein Unstained Standards; lane 2, soluble lysate; lane 3, flowthrough; lane 4, wash; lane 5, purified product.

Section 7
Scaling Up

EconoFit Columns are available in 1 and 5 ml formats. The Profinity eXact Resin is also available in 10 ml bottles. For quick scale-up, two or more columns may 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. Ask your local Bio-Rad representative or go to **bio-rad.com/ResinsandColumns** for more information.

Section 8
Regenerating, Cleaning, and Storage

Protein cross-contamination, frit clogging, and increased backpressure may result from column overuse. It is recommended to dispose of a column after five purifications. However, the following regeneration procedure may be used to prolong the useful lifespan of a column. In addition, in order to avoid cross-contamination it is recommended that single columns be designated for single proteins. In order to reuse a column, the Profinity eXact tag, which is bound tightly to the functional ligand, MUST be removed. Therefore, to maintain good column flow properties, and to prepare the column for a subsequent purification, it is recommended that the column be regenerated after each use. For the 1 ml columns, run the regeneration protocol at 3 ml/min. For the 5 ml columns, run the regeneration protocol at 10 ml/min.

Regeneration and Storage

1. After elution of the purified protein, wash the column with 5 CV of bind/wash buffer.
2. Regenerate the column with 3 CV of 100 mM phosphoric acid.
3. Neutralize the column with 5 CV of bind/wash buffer.

Note: The resin may also be cleaned with 0.1 M NaOH. Limit exposure time to 3 hours and immediately neutralize with 5 CV of bind/wash buffer. Regeneration of the resin with phosphoric acid is still necessary, as NaOH alone will not efficiently remove the Profinity eXact tag from the resin.

Section 9
Troubleshooting Guide

Table 9. Troubleshooting guide.

Problem	Possible Cause	Solution
Column clogging or slow flow rate	Particulates in samples or buffers	Filter all samples and buffers through 0.45 µm filter prior to application
	Sample too viscous	Add nuclease to lysate to degrade DNA
No target protein in eluate	Low level of target protein in starting material	Check expression level by SDS-PAGE
	Target protein not binding	Used resin must be regenerated before reuse. Follow regeneration protocol in section 8
	Inaccessible tag	Clone into pPaL's Spe I site
Target protein in flow-through or wash fractions	Intrinsic cleavage	Ensure no chloride ions are present in lysate or bind/wash buffers. Perform purification at 4°C. Clone into pPAL's Spe I site
Incomplete elution	No or slow cleavage	Lengthen cleavage incubation step. Clone into pPAL's Spe I site

Section 10
Ordering Information

Catalog #	Description
EconoFit Profinity eXact Columns	
12009292	**EconoFit Profinity eXact Column**, 1 x 1 ml column
12009293	**EconoFit Profinity eXact Columns**, 5 x 1 ml columns
12009294	**EconoFit Profinity eXact Column**, 1 x 5 ml column
Profinity eXact Resins	
1563005	**Profinity eXact Purification Resin**, 10 ml bottle

Section 11
Bibliography

Ruan B et al. (2004). Engineering subtilisin into a fluoride triggered processing protease useful for one-step purification. Biochemistry 43, 14,539–14,546.

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