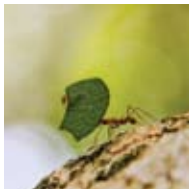


AFFINITY PURIFICATION SYSTEMS

Profinity eXact™ Purification Resin

A Novel Tag System for the Purification and Processing of Fusion-Tagged Proteins

Introduction

Research on the structure and function of proteins includes the expression, purification, detection, and analysis of biologically significant and clinically relevant proteins. Consequently, the number of proteins produced using recombinant techniques has increased dramatically. Due to its ease of use and high specificity, affinity chromatography continues to be the main avenue for purification of fusion-tagged proteins.

Profinity eXact purification resin (Figure 1) is part of the Profinity eXact fusion-tag system, a patent-pending *E. coli*-based expression and purification system that simplifies purification and tag removal of fusion-tagged proteins. Typically, complications in removing the tag and protease are unavoidable, adding enormous time and reagent costs to the purification process.

Fig. 1. Profinity eXact purification resin and consumables. For single-step purification and cleavage of fusion-tagged proteins, Profinity eXact resin is available in bottles and in convenient prepacked spin columns and cartridges.

With cleavage incubation times occurring in as little as 30 minutes, the Profinity eXact fusion-tag system offers customers a novel alternative to purify and process their fusion proteins in a single step, without the addition of proteases.

Profinity eXact Technology

The Profinity eXact fusion-tag system utilizes a modified form of subtilisin protease, immobilized on a chromatographic support, that can generate pure, tag-free target protein in a single step. The tag in this system is the prodomain of the subtilisin protease, a 75-amino acid sequence that fuses to the N-terminus of a target protein of interest. The prodomain and mature subtilisin protease sequences have been coengineered to produce a specific, high-affinity interaction between the binding partners. A major advantage of this system is that the subtilisin protease not only binds and recognizes the tag, but also quickly and precisely cleaves the tag from the fusion protein to release the purified target protein. At the end of the purification process, the tag remains tightly bound to the resin (Figure 2).

BIO-RAD

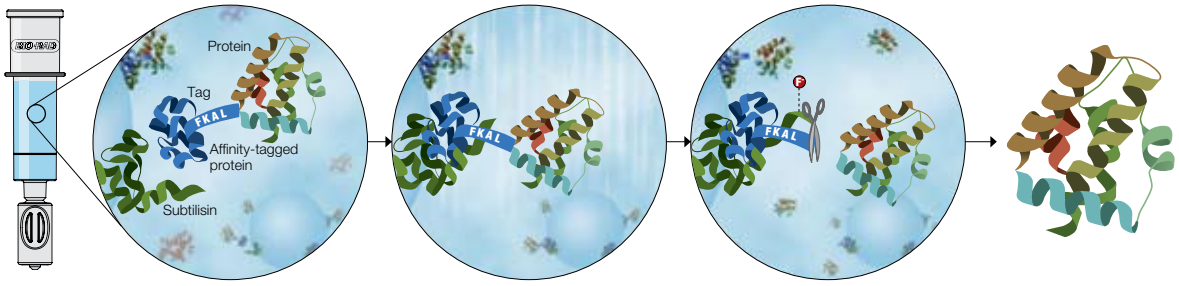


Fig. 2. Purification and on-column tag cleavage using the Profinity eXact fusion-tag system. During sample application, immobilized subtilisin protease (ligand) recognizes and binds the affinity-tagged protein. Washing the column removes unbound contaminants. Application of fluoride-containing elution buffer triggers subtilisin to quickly and precisely cleave the tag from the fusion protein after the FKAL cleavage recognition sequence. The tag remains tightly bound to the resin, and a highly purified protein with only its native amino acid sequence is released.

Resin Characteristics

Profinity eXact purification resin consists of a Superflow agarose bead, which uses the modified subtilisin protease as its ligand. Fusion-tag proteins expressed using Profinity eXact pPAL7 expression vectors contain the prodomain sequence, or tag, of the subtilisin protease, resulting in a highly stringent mechanism of interaction between the two. The Superflow base matrix provides high mechanical stability to withstand the pressure limits of low-to-medium column chromatography systems.

Elution of tag-free protein is triggered by the addition of fluoride-containing buffers. Azides and chlorides also cause cleavage of tagged proteins, so these ions should be avoided in the lysis, binding, and washing steps of the purification protocol.

Profinity eXact resin is stable across a pH range of 2–13 and is compatible with many reagents used for purification of tagged proteins. It can be used under native and, if desired, denaturing conditions of up to 4 M urea. The stability of Profinity eXact purification resin permits the use of a range of reagents, including detergents, reducing agents, buffering agents, and a variety of additives (Table 1).

Table 1. Profinity eXact purification resin chemical compatibility.*

Lysis solutions	Bacterial lysis and extraction reagent (Bio-Rad) B-PER protein extraction reagent in phosphate buffer (Pierce Biotechnology, Inc.) B-PER protein extraction reagent in Tris buffer* (Pierce) BugBuster protein extraction reagent** (Novagen, Inc.) FastBreak cell lysis reagent** (Promega Corporation)
Protease inhibitors	1x protease inhibitor cocktail (BD Biosciences Pharmingen) 2x protease inhibitor cocktail set 1 (Merck UK: Calbiochem) Complete protease inhibitor cocktail tablets (Roche) 0.5 mM PMSF 0.1 mM TLCK 0.1 mM TPCK
Detergents	5% (v/v) Triton X-100 5% (v/v) NP-40 5% (v/v) Tween 20 5% (w/v) octylthioglucoside 5% (w/v) n-dodecyl β-D-maltoside 5% (w/v) CHAPS 5% (w/v) CHAPSO
Reducing agents	20 mM β-mercaptoethanol 10 mM DTT 5 mM TCEP
Chelating reagents	20 mM EDTA 20 mM EGTA
Buffer reagents	50 mM Tris-acetate, pH 7.2 50 mM Tris-phosphate, pH 7.2 50 mM HEPES, pH 7.2 50 mM PIPES, pH 7.2 50 mM MOPS, pH 7.2 50 mM MES, pH 7.2
Additives	20% (v/v) glycerol 20% (v/v) ethylene glycol 20% (v/v) ethanol 20% (w/v) sorbitol 20% (w/v) sucrose 200 mM imidazole 200 mM sodium acetate 100 mM sodium borate 100 mM sodium citrate 100 mM sodium sulfate 15% (w/v) ammonium sulfate 5% (v/v) DMSO 5 mM $MgCl_2$** 5 mM $CaCl_2$**

* Compatibility determined using Profinity eXact control lysate; some reagents, like ammonium sulfate, are protein-dependent.

** Chloride ions trigger cleavage of target proteins.

Performance Benefits

Traditional methods of affinity tag cleavage necessitate the addition of a protease with prolonged or overnight incubations, and are then followed by subsequent chromatography steps that subtract the target protein from the tag and the cleavage enzyme. Incubation times with endoproteases typically range from between 16 hours to as long as 48 hours. The novel Profinity eXact fusion-tag system generates a highly purified, recombinant protein, containing only its native amino acid sequence, in a single step and in a fraction of the time compared to other methods. The time and cost savings make the system useful in both academic and industrial settings.

System features include:

- Single-step purification and tag cleavage
- Delivery of proteins with ultrahigh purity
- Protease addition not required

Specifications

Functional ligand	Modified subtilisin protease (27.8 kD)
Base bead	Superflow agarose (6% cross-linked)
Form	50% suspension in 100 mM potassium phosphate, 0.02% sodium azide, pH 7.2
Particle size	60–160 µm
Dynamic binding capacity*	>3 mg tag-free maltose binding protein (MBP)/ml resin
Maximum flow rate recommended	1,000 cm/hr at 25°C linear flow rate**
pH stability	2–13
Chemical compatibility	See Table 1
Storage	4°C
Shelf life in 20% ethanol	>1 year at 4°C
Operational temperature	4–40°C
Column volumes	
Spin	100 µl
Cartridge	1 ml, 5 ml
Bind/wash buffer	0.1 M sodium phosphate, pH 7.2
Elution buffer	0.1 M sodium phosphate, 0.1 M sodium fluoride, pH 7.2

* Dynamic binding capacity determined using conditions described in Figure 3 (dynamic binding capacity varies from protein to protein).

** Recommended linear flow rate determined by the following method: Flow rate of 100 mM sodium phosphate, pH 7.2 was incrementally increased through a 1 ml Bio-Scale Mini Profinity eXact cartridge. The pressure-flow curve for Profinity eXact purification resin became nonlinear at linear velocities above 1,000 cm/hr, with a 20% compression factor.

Tag-Free Protein in a Single Step

Protease immobilization on a chromatography support allows parallel purification and on-column processing of fusion-tagged proteins, distinguishing Profinity eXact purification resin from traditional methods of tag cleavage. Researchers can obtain high-efficiency tag cleavage and generate target proteins with a native N-terminus in as little as 60 minutes (includes 30 minutes cleavage incubation time). The resulting purity of the eluted protein is typically higher than for any other affinity tag system, due to specific recognition of the prodomain sequence by subtilisin and to high fidelity of the protease reaction.

Figure 3 illustrates single-step purification of Green Fluorescent Protein (GFP) and MBP expressed with the Profinity eXact tag. Sample lysates were loaded on 1 ml Bio-Scale Mini Profinity eXact cartridges and run on the BioLogic DuoFlow™ chromatography system. Minimal to zero uncleaved target protein is found in elution lanes, indicating high cleavage efficiency.

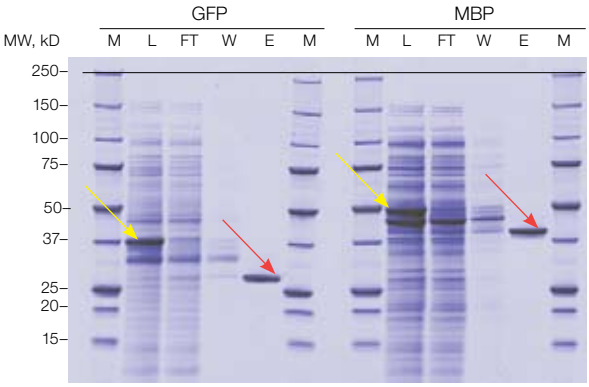


Fig. 3. SDS-PAGE analysis of proteins, GFP (26 kD) and MBP (40 kD), fused to Profinity eXact purification resin and purified by Bio-Scale Mini Profinity eXact cartridges. Crude *E. coli* lysate containing the expressed proteins (2 ml) was loaded onto a 1 ml Bio-Scale Mini Profinity eXact cartridge on a BioLogic DuoFlow system with binding/wash buffer at 1 ml/min. The cartridge was washed with 10 column volumes (CV) of the same buffer at 1 ml/min. Proteins were eluted with 3 CV potassium fluoride buffer at 0.1 ml/min for 30 min at room temperature. Total purification time required to generate tag-free proteins without addition of protease was approximately 60 min. M, markers (Precision Plus Protein™ standards); L, load (crude lysate); FT, flowthrough; W, wash; E, eluate. (➝), tagged proteins; (➝), tag-free purified proteins.

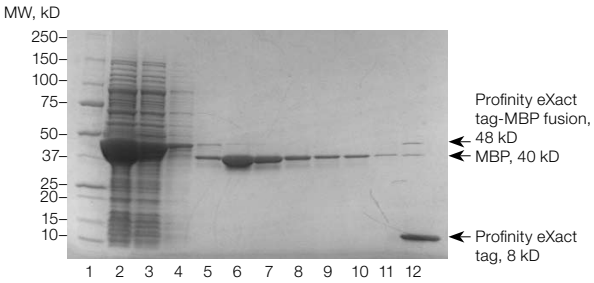
No Addition of Protease

Affinity tag removal is required whether a protein is used for structural analysis or for treatment in a clinical setting. It can be a daunting task, especially in large-scale purification processes, to effectively remove the tag, protease, and undesirable cleavage products that result from lengthened incubation times. These problems can result in diminished purity levels, reduced yields, and significantly increased protocol time and costs.

A comparative study demonstrates the advantages of the Profinity eXact system over conventional methods of cleavage (Figure 4). An MBP fusion was constructed into a modified vector encoding both a GST-tag and a TEV recognition cleavage sequence upstream of the MBP sequence. Details of MBP construct generation can be found in bulletin 5652. Prior to purification, optimization experiments of the cleavage reaction conditions (enzyme-substrate ratio and proteolytic incubation time) were performed. Purification of the GST-tagged MBP fusion was carried out using standard affinity chromatography on a GSTrap column, and target protein was eluted with glutathione. The eluate was then dialyzed to remove glutathione. The GST-tag was cleaved from the MBP protein by the addition of AcTEV protease during dialysis and was then passed through a GSTrap column to remove the GST-tag. Tag-free MBP was finally obtained by running the protease mixture over a new HisTrap FF column to remove the AcTEV enzyme. Total purification/cleavage time was approximately 20 hours and did not include the time spent on optimizing protease concentration and incubation time (Figure 4).

For purification with the Profinity eXact fusion-tag system, an MBP fusion protein was constructed using the Profinity eXact pPAL7 RIC-ready expression vector by restriction-independent cloning (RIC). Details of cloning and mutagenesis procedures are available in bulletin 5652. Expressed MBP was both purified and processed by on-column cleavage. Protocol steps included lysate load, wash, and elution of target protein. Addition of potassium fluoride triggered subtilisin's processing activity, resulting in elution of tag-free target in a single step. In contrast to purification with GST-TEV, purification and cleavage with the Profinity eXact system required approximately 60 minutes and did not require protease addition or optimization steps.

A. Profinity eXact tag-MBP fusion; total time: 60 min (tag free)



B. GST-TEV-MBP fusion; total time: 20 hours (tag free)

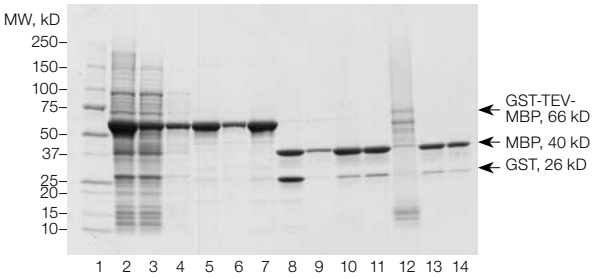


Fig. 4. Comparison of cleavage using the Profinity eXact fusion tag vs. a GST-TEV tag. A, MBP purification using Profinity eXact tag. Lane 1, Precision Plus Protein unstained standards; lane 2, lysate; lane 3, flowthrough; lane 4, wash; lanes 5–11, tag-free MBP in elution fractions; lane 12, Profinity eXact tag (~8 kD) stripped from the column using 0.1 M phosphoric acid; **B**, GST-TEV-MBP fusion protein purification and cleavage with TEV protease. After cleavage, the GST and MBP mixture was passed through a GSTrap column to bind cleaved GST. Collected flowthrough with tag-free MBP was loaded onto a HisTrap FF column to remove His-tagged AcTEV; MBP was collected in the flowthrough fraction. Lane 1, Precision Plus Protein unstained standards; lane 2, lysate; lane 3, flowthrough; lane 4, wash; lanes 5–7, fractions containing GST-TEV-MBP fusion protein; lane 8, cleaved GST-TEV-MBP fusion protein; lanes 9–12, purified MBP, flow-through fractions from GSTrap column; lane 13, pooled fractions (lanes 9–12); lane 14, MBP from flowthrough of HisTrap FF column.

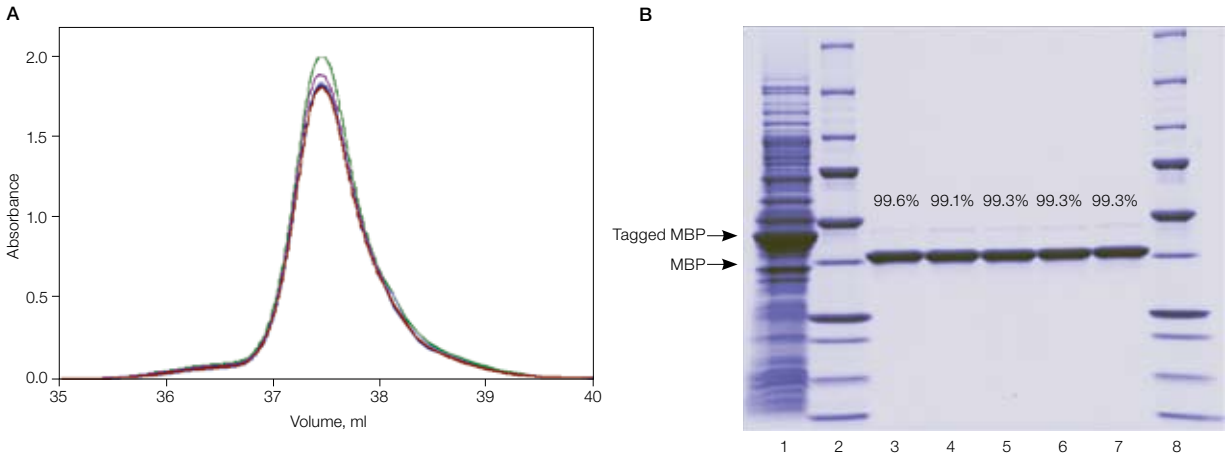


Fig. 5. Stability and reusability of Profinity eXact purification resin. A, overlaid chromatograms of MBP purification with a 1 ml Bio-Scale Mini Profinity eXact cartridge on the BioLogic DuoFlow system. Binding capacities for cycles 1 to 5 ranged from 3.7 mg/ml to 3.3 mg/ml; **B**, SDS-PAGE analysis of MBP protein eluted from cartridge cycling studies using Criterion™ 4-20% Tris-HCl gels. Lane 1, *E. coli* lysate containing tagged MBP protein; lane 2, Precision Plus Protein standards; lanes 3–7, 3 µg of tag-free MBP protein eluted from cartridge cycling studies; lane 8, Precision Plus Protein standards. Percentage purity of proteins in lanes 3–7 is indicated.

Increased Stability for Reproducible Results

Profinity eXact purification resin can be reused without altering the quality or performance of the resin. In an experiment testing the stability of the resin, a 1 ml Bio-Scale Mini Profinity eXact cartridge was used to purify the tagged MBP protein for 5 consecutive cycles. The sample was loaded and washed, and tag-free protein was eluted from the cartridge. The Profinity eXact tag was retained with the immobilized subtilisin protease during elution and then stripped off the resin with 0.1 M phosphoric acid. After reequilibrating the cartridge, subsequent bind, wash, elute, and strip steps were carried out for a total of five cycles (Figure 5).

Results demonstrate that consistent and reproducible separation can be obtained, with minimal compromise to yield and negligible effect on purity. Quantitative analysis shows that Profinity eXact purification resin was able to retain 90% of its original capacity after five cycles of use. Yield of protein obtained from cycles 1 to 5 ranged from 3.7 mg/ml to 3.3 mg/ml.

Resin Availability

Profinity eXact purification resin is available in 10 ml bottles and in prepacked cartridges and spin columns. Purifications can be carried out on liquid chromatography systems using both gravity flow and spin columns. The resin is supplied in 100 mM sodium phosphate, pH 7.2, containing 0.02% sodium azide.

Bottled Profinity eXact Purification Resin

Profinity eXact purification resin is supplied as a 50% suspension in 10 ml bottles and may be used in a variety of purification formats. Bio-Rad's selection of columns includes glass Econo-Column® chromatography columns for gravity flow and low-pressure purification, Bio-Scale™ MT high-resolution columns for medium-pressure purifications, and empty Econo-Pac® and Poly-Prep® columns for gravity-flow purifications.

For more information regarding Bio-Rad's complete line of empty chromatography columns and accessories, request bulletin 2289.

Bio-Scale Mini Profinity eXact Cartridges

Bio-Scale Mini Profinity eXact cartridges deliver high-performance purification of tag-free protein in convenient 1 and 5 ml formats. Cartridges can be used seamlessly with BioLogic™ chromatography systems, the Profinia™ protein purification system, a peristaltic pump, or with appropriate column adaptors with any chromatography system.

All Bio-Scale Mini cartridges contain built-in luer fittings, allowing simple operation with a syringe for those with limited access to chromatography instruments (Figure 6).

Fig. 6. Syringe purification using a Bio-Scale Mini Profinity eXact cartridge and poly column rack.

Profinity eXact Mini Spin Columns

Profinity eXact mini spin columns are ideal for rapid expression, screening, and optimization of affinity-tag purification procedures. The newly designed column features an innovative end cap. On first use, the end cap is snapped off to drain the shipping buffer. When reversed, it plugs the column during binding (incubation optional) and elution incubation steps. Profinity eXact mini spin columns are available individually or in the following two kit formats:

- Profinity eXact mini spin purification starter kit—includes spin columns, collection tubes, lyophilized control lysate, purification buffers
- Profinity eXact expression and purification starter kit—includes Profinity eXact cloning and expression starter kit and Profinity eXact mini spin purification starter kit

Ordering Information

Catalog #	Description
156-3000	**Profinity eXact Cloning and Expression Starter Kit**, includes 25 µl of 20 ng/µl RIC-ready pPAL vector, 100 µl of 100 ng/µl supercoiled pPAL vector, chemi-competent cells, SOC growth media, 20 reactions
156-3001	**Profinity eXact pPAL RIC-Ready Expression Vector Kit,** includes 25 µl of 20 ng/µl vector, 20 reactions
156-3002	**Profinity eXact pPAL Supercoiled Expression Vector Kit,** includes 100 µl of 100 ng/µl vector, 20 reactions
156-3003	**BL21(DE3) Chemi-Competent Expression Cells**, includes 10 x 0.05 ml BL21(DE3) cells, pUC19 control plasmid, 10 ml vial of SOC growth media
156-3004	**Profinity eXact Antibody Reagent**
156-3005	**Profinity eXact Purification Resin**, 10 ml
156-3006	**Profinity eXact Mini Spin Purification Starter Kit**, includes 10 spin columns, 2 ml capped tubes (10), 2 ml capless tubes (10), lyophilized control protein lysate, bacterial lysis reagent, 50 ml bind/wash buffer, 20 ml elution buffer
156-3007	**Profinity eXact Mini Spin Columns**, includes 10 spin columns, 2 ml capped tubes (10), and 2 ml capless tubes (10)
156-3008	**Profinity eXact Expression and Purification Starter Kit**, includes 1 Profinity eXact cloning and expression starter kit (20 reactions) and 1 Profinity eXact mini spin purification starter kit (10 spin columns)
732-4646	**Bio-Scale Mini Profinity eXact Cartridges**, 2 x 1 ml
732-4647	**Bio-Scale Mini Profinity eXact Cartridges**, 4 x 1 ml
732-4648	**Bio-Scale Mini Profinity eXact Cartridges**, 1 x 5 ml
Colorimetric Detection Kits	
170-8235	**Opti-4CN Substrate Kit**
170-8237	**Opti-4CN Goat Anti-Mouse Detection Kit**

BIO-RAD

Bio-Rad Laboratories, Inc.

Life Science Group

Web site *www.bio-rad.com* ***USA*** *800 4BIORAD* ***Australia*** *61 02 9914 2800* ***Austria*** *01 877 89 01* ***Belgium*** *09 385 55 11* ***Brazil*** *55 21 3237 9400* ***Canada*** *905 364 3435* ***China*** *86 21 6426 0808* ***Czech Republic*** *420 241 430 532* ***Denmark*** *44 52 10 00* ***Finland*** *09 804 22 00* ***France*** *01 47 95 69 65* ***Germany*** *089 318 84 0* ***Greece*** *30 210 777 4396* ***Hong Kong*** *852 2789 3300* ***Hungary*** *36 1 455 8800* ***India*** *91 124 4029300* ***Israel*** *03 963 6050* ***Italy*** *39 02 216091* ***Japan*** *03 6361 7000* ***Korea*** *82 2 3473 4460* ***Mexico*** *52 555 488 7670* ***The Netherlands*** *0318 540666* ***New Zealand*** *0508 805 500* ***Norway*** *23 38 41 30* ***Poland*** *48 22 331 99 99* ***Portugal*** *351 21 472 7700* ***Russia*** *7 495 721 14 04* ***Singapore*** *65 6415 3188* ***South Africa*** *27 861 246 723* ***Spain*** *34 91 590 5200* ***Sweden*** *08 555 12700* ***Switzerland*** *061 717 95 55* ***Taiwan*** *886 2 2578 7189* ***United Kingdom*** *020 8328 2000*