Profinity eXact™
Fusion-Tag System

Affinity Tag Purification and Tag Removal

BIO-RAD

Fast Forward to Tag-Free Protein

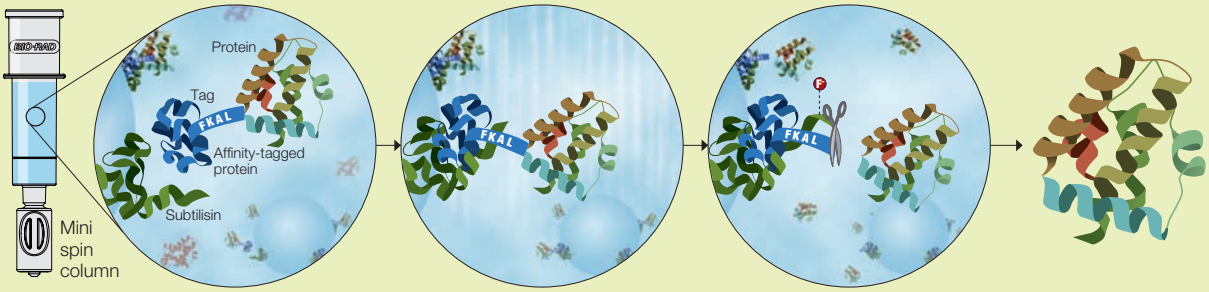
Protein fusion technology has greatly simplified the process of purifying recombinant proteins. Recombinant technology has made the expression of large numbers of proteins possible, usually in tagged format, allowing their use in standard chromatography applications.

The Profinity eXact fusion-tag system addresses the bottlenecks in the tag-removal process during affinity-tagged protein purification. The Profinity eXact system is a novel *E. coli*-based system for the expression, detection, purification, and on-column cleavage of affinity-tagged proteins, without the addition of protease. The system offers single-step purification without the hassle and expense of cleavage enzymes, incubation times, or removal of reagents.

Key Features

- Purification and processing of fusion-tagged proteins in a single step
- On-column cleavage in as little as 30 minutes
- Elimination of protease-addition step
- Precise cleavage at the N-terminus to generate native protein sequence

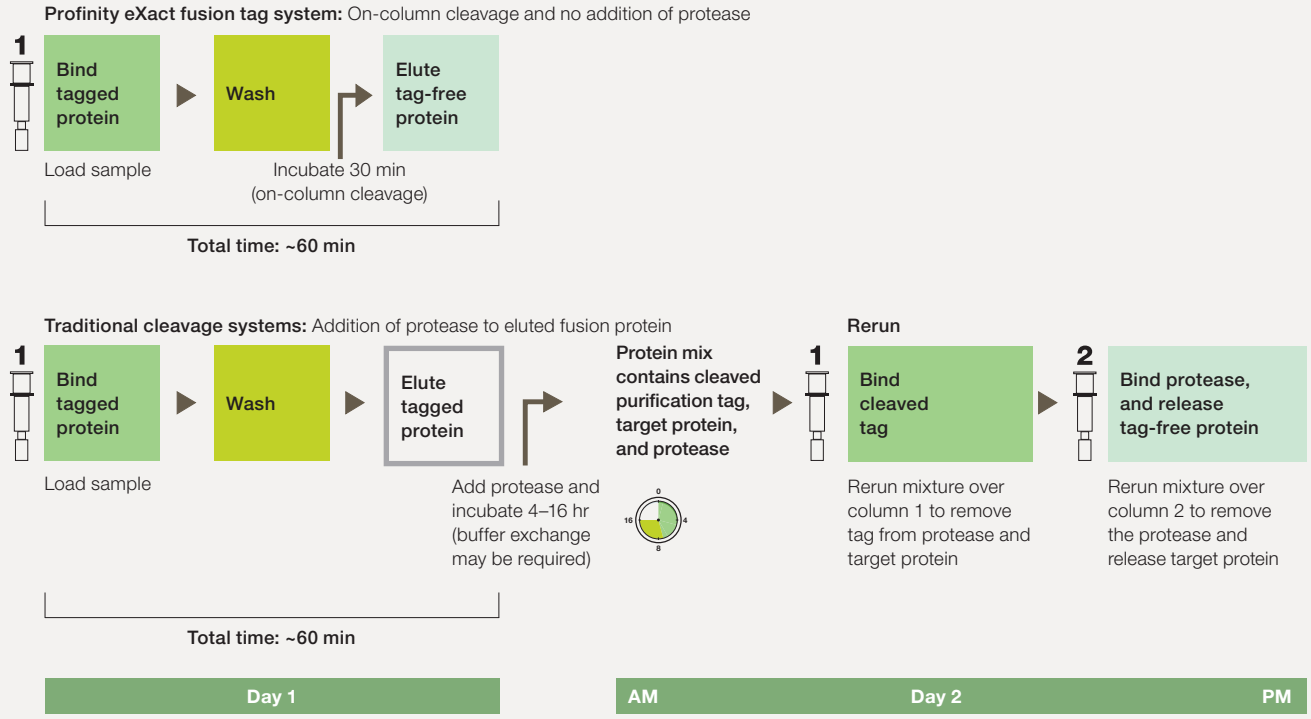
Profinity eXact fusion-tag system consists of expression vectors, chemi-competent cells, SOC growth media, spin columns, purification resin, cartridges, and detection reagents.



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 cleavage recognition sequence ending in FKAL. The tag remains tightly bound to the resin, and a highly purified protein with only its native amino acid sequence is released.

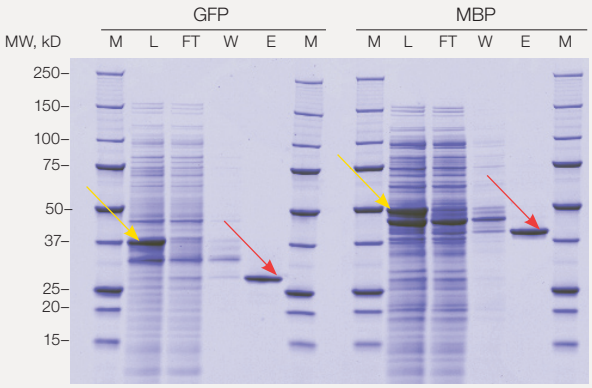
One-Step Purification and Tag Cleavage

In experiments requiring tag cleavage, protease and tag removal problems are unavoidable. The Profinity eXact fusion-tag system easily overcomes these challenges with parallel purification and on-column cleavage. Availability of immobilized protease in the column appreciably shortens the purification process.



Precise Cleavage and High Purity

Immobilized protease on a chromatography support is unique to Profinity eXact purification resin, distinguishing it from traditional methods of tag cleavage. Purity of the eluted protein is typically higher than for other affinity tag systems due to the specific recognition of the immobilized subtilisin protease for its tag sequence and the high fidelity of the protease reaction.



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 (2 ml) containing the expressed proteins 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.

Ordering Information

Catalog #	Description
Profinity eXact Fusion-Tag System	
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
156-3000	**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, SOC growth media, 20 reactions
156-3005	**Profinity eXact Purification Resin**, 10 ml
156-3007	**Profinity eXact Mini Spin Columns**, includes 10 spin columns, 2 ml capped tubes (10), and 2 ml capless tubes (10)
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
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)
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
Profinity eXact Detection Reagent	
156-3004	**Profinity eXact Antibody Reagent**
Colorimetric Detection Kits	
170-8235	**Opti-4CN Substrate Kit**
170-8237	**Opti-4CN Goat Anti-Mouse Detection Kit**

Catalog #	Description
Profinia™ Instruments	
620-1004	**Profinia Instrument With Accessory Kit**, 100–240 V, includes cleaning tray, inline filter pack, 2 x 50 ml sample lids, 2 x 15 ml sample lids, bottle starter pack, waste/diluent bottle set
620-1005	**Profinia Instrument With Accessory Kit and Native IMAC Starter Kit**, 100–240 V, includes cleaning tray, inline filter pack, 2 x 50 ml sample lids, 2 x 15 ml sample lids, bottle starter pack, waste/diluent bottle set, Profinia native IMAC buffer kit, 1 x 1 ml IMAC and 1 x 10 ml desalting cartridge, *E. coli* lysate
620-1006	**Profinia Instrument With Accessory Kit and GST Starter Kit**, 100–240 V, includes cleaning tray, inline filter pack, 2 x 50 ml sample lids, 2 x 15 ml sample lids, bottle starter pack, waste/diluent bottle set, Profinia GST buffer kit, 1 x 1 ml GST and 1 x 10 ml desalting cartridge, *E. coli* lysate, glutathione reagent
Ready-to-Use Affinity Media	
156-0131	**Profinity™ IMAC Ni-Charged Resin**, 10 ml
156-0133	**Profinity IMAC Ni-Charged Resin**, 25 ml
156-0135	**Profinity IMAC Ni-Charged Resin**, 100 ml
156-0137	**Profinity IMAC Ni-Charged Resin**, 500 ml
156-0121	**Profinity IMAC Uncharged Resin**, 10 ml
156-0123	**Profinity IMAC Uncharged Resin**, 50 ml
156-0125	**Profinity IMAC Uncharged Resin**, 500 ml
156-0127	**Profinity IMAC Uncharged Resin**, 1 L
Bio-Scale Mini Affinity and Desalting Cartridges	
732-4610	**Bio-Scale Mini Profinity IMAC Cartridges**, 5 x 1 ml
732-4612	**Bio-Scale Mini Profinity IMAC Cartridge**, 1 x 5 ml
732-4614	**Bio-Scale Mini Profinity IMAC Cartridges**, 5 x 5 ml
732-4620	**Bio-Scale Mini Profinity GST Cartridges**, 5 x 1 ml
732-4622	**Bio-Scale Mini Profinity GST Cartridge**, 1 x 5 ml
732-4624	**Bio-Scale Mini Profinity GST Cartridges**, 5 x 5 ml
732-5304	**Bio-Scale Mini Bio-Gel® P-6 Desalting Cartridges**, 5 x 10 ml
732-5312	**Bio-Scale Mini Bio-Gel P-6 Desalting Cartridge**, 1 x 50 ml
732-5314	**Bio-Scale Mini Bio-Gel P-6 Desalting Cartridges**, 5 x 50 m

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