Profinity eXact™ Mini Spin Columns 156-3007

10 x 0.6 ml of 17% slurry of Profinity eXact purification resin (0.1 ml bed volume)

For research use only

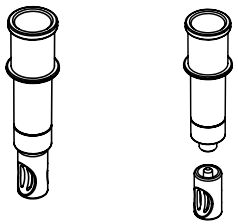
Storage and Stability

The Profinity eXact mini spin columns are stable for 1 year when stored at 4°C.

Kit Contents

10 x 0.6 ml of 17% slurry of Profinity eXact purification resin in 100 mM sodium phosphate, 0.02% (w/v) NaN_3, pH 7.2.

50 x 2 ml collection tubes.



Snap off end cap to use. When reversed, the end cap plugs the column during incubation steps. Carry out purification according to protocol.

Description

Profinity eXact mini spin columns are pre-packed, single-use columns for rapid purification and in-line tag removal of Profinity eXact-tagged proteins by affinity chromatography. The spin columns can be used with a standard centrifuge and one purification run takes less than one hour.

The Profinity eXact purification resin contained in the columns is a novel affinity medium immobilized with a modified version of the subtilisin protease. The highly specific protease binds strongly to the Profinity eXact tag with sub-nanomolar affinity. It also acts as a processing protease under controllable conditions to cleave and elute the tag-free target protein from the column with its native or desired N-terminal amino acids. The result of this simple process is a true, single-step affinity purification and tag removal procedure. The novel resin has high protein binding capacity and excellent compatibility with a wide range of reagents, buffers, and additives. Please see the tables for properties and compatibilities of the Profinity eXact mini spin columns.

Table 1. Properties of Profinity eXact mini spin columns

Column material	Polypropylene barrel and polyethylene frits
Base bead	Superflow agarose (6% cross-linked)
Functional ligand	Modified subtilisin protease (27.8 kD)
Bead size	60–160 µm
pH stability	pH 2–13
Column bed volume	0.1 ml
Binding capacity	> 300 µg tag-free protein/column*
Chemical compatibility	Stable in most commonly used buffers, detergents, reducing agents and additives (see table 2)
Avoid before elution	F^-, Cl^-, azide, nitrite, formate and ionic detergents
Storage	4°C in 100 mM sodium phosphate, 0.02% (w/v) sodium azide, pH 7.2
Shelf life	> 1 year at 4°C
Operational temperature	4–40°C
Bind/Wash buffer	100 mM sodium phosphate, pH 7.2
High stringency bind/wash buffer	Optional, 100 mM sodium phosphate, up to 2 M sodium acetate, pH 7.2
Elution buffer	100 mM sodium phosphate, 100 mM sodium fluoride, pH 7.2

* Binding capacity determined using Profinity eXact MBP control lysate and is protein dependent.

Protocol Notes

1. **Avoid chloride (NaCl, KCl, or Tris-HCl) and azide containing buffers before the elution step is performed. Fluoride, chloride (weak trigger), and azide ions can prematurely trigger the cleavage of the Profinity eXact tag, resulting in loss of column capacity and target protein yield.**
2. All centrifugations are performed at 1,000 x g for 30 sec on a table-top standard centrifuge.
3. For best results, pre-chill binding and wash buffers on ice prior to use.

Quick Purification Protocol (see Profinity eXact Purification System Manual for Complete Protocol)

Column pre-equilibration

1. Thoroughly re-suspend the resin, snap off end cap of spin column, and retain cap for later use. Place in a 2 ml collection tube and spin. Decant filtrate and replace spin column in collection tube.
2. Pre-equilibrate resin with 500 µl (5 CV) ice-cold bind/wash buffer. Spin and discard filtrate.
3. Repeat step 2.

Sample binding

4. Attach end cap to column, add up to 600 µl of clear *E. coli* lysate containing Profinity eXact-tagged protein, and attach top cap.
5. Gently mix 5–20 min at 4°C on a rocking platform or rotator.

   Note: For quick screening, incubate the lysate and resin at room temperature for 1–5 min. Purification of larger proteins may benefit from longer incubations at 4°C.
6. Carefully twist and remove end cap. **Retain end cap**, place column in a new 2 ml collection tube, spin, and collect the flow through.

Washing

7. Place column in a new 2 ml collection tube, add 500 µl (5 CV) of bind/wash buffer, and re-suspend resin by pipetting 3 times.
8. Spin and collect the wash fraction.
9. Repeat steps 7 and 8.

Elution

10. Attach end cap, add 500 µl (5 CV) of elution buffer, attach the top cap, and gently mix 30 min at room temperature on a rocking platform or rotator.

    Note: Shorter incubation time may result in incomplete cleavage of the Profinity eXact tag from the fusion protein leading to reduced yields.
11. Carefully twist and remove end cap. Place column in a new 2 ml collection tube, and spin to obtain tag-free target protein in the eluate.
12. Analyze samples collected after the bind (flow through), wash and elution steps as appropriate.

Table 2. Chemical compatibilites of Profinity eXact mini spin columns[†]	
Lysis solutions	Bio-Rad bacterial lysis and extraction reagent
	Pierce B-PER protein extraction reagent in Pi buffer
	Pierce B-PER protein extraction reagent in Tris buffer*
	Novagen BugBuster protein extraction reagent*
	Promega FastBreak cell lysis reagent*
Protease inhibitors	1x BD Biosciences Pharmingen protease inhibitor cocktail
	2x CalBioChem protease inhibitor cocktail set 1
	Roche complete protease Inhibitor tablets
	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 reagents	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 $CaCl_2$*

[†] Compatibilities determined using Profinity eXact control lysate; some reagents, like ammonium sulfate, are protein dependent.

* Note: Chloride ions trigger cleavage of target proteins from the column.

Related Products

Catalog#	Description
156-3005	**Profinity eXact Purification Resin**, 10 ml
156-3006	**Profinity eXact Mini Purification Starter Kit**, 10 pk
156-3007	**Profinity eXact Mini Spin Columns**, 10 pk
156-3008	**Profinity eXact Expression and Purification Starter Kit**
732-4646	**Bio-Scale Mini Profinity eXact Cartridges**, 2 x 1 ml
732-4648	**Bio-Scale Mini Profinity eXact Cartridge**, 1 x 5 ml

Legal Information

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