ProTEV Protease for Fusion Protein Processing

ABSTRACT ProTEV Protease is an improved 50 kDa version of the NIa protease from tobacco etch virus (TEV) that has been engineered to be more stable than native TEV protease for prolonged enzyme activity (1–3). It is used primarily to cleave affinity tags from fusion proteins during or after affinity purification. ProTEV Protease is active over a broad pH and temperature range to allow tailoring of cleavage conditions for the fusion protein of interest.

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Rarely found in proteins, the six-amino-acid recognition sequence of ProTEV Protease makes the enzyme highly specific for the cleavage site.

INTRODUCTION

Many proteins are expressed as fusion partners with affinity tags, such as glutathione-S-transferase (GST) or maltose binding protein (MBP), to selectively bind the proteins using affinity purification resins. While such resins yield high-purity protein quickly, the large affinity tags are undesirable for some downstream applications. Most expression vectors are designed with a specific protein cleavage site between the two fusion partners to remove the affinity tag after purification.

Proteases such as Factor Xa (FXa; Cat.# V5581) are commonly used for removing affinity tags. The main disadvantage of FXa is that its four-amino-acid recognition sequence is commonly found in proteins. As a result, FXa may cleave the protein of interest while removing the affinity tag. Unlike FXa, ProTEV Protease recognizes a rare amino acid sequence, EXXYXQ, where X is any amino acid and cleavage occurs after the glutamine residue (4,5). TEV protease will cleave proteins with 19 of the 20 amino acids present after the glutamine residue; the exception is proline (6). Cleavage sites for TEV protease are found in many vectors including the pFN2A and pFN2K (GST) Flexi® Vectors (Cat.# C8461 and C8471).

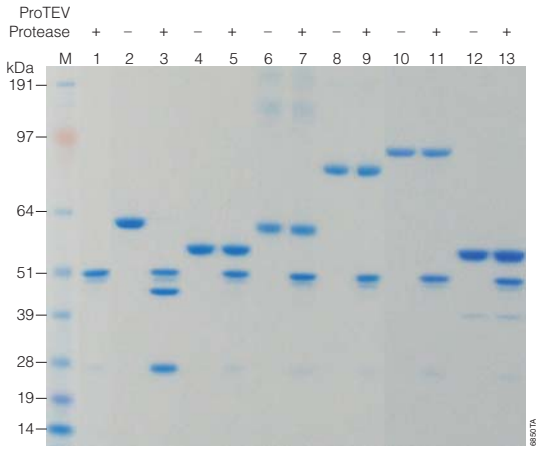

Figure 1. ProTEV Protease has highly specific proteolytic activity. Fifteen micrograms of six different proteins were incubated with or without 10 units of ProTEV Protease for 72 hours at 30 °C. Digestion of the proteins was analyzed by SDS-PAGE and stained with SimplyBlue™ SafeStain (Invitrogen). Lane M, SeeBlue® Plus2 (Invitrogen); lane 1, ProTEV Protease alone; lanes 2,3, GST-MBP with TEV protease site; lanes 4,5, GST-HaloTag® fusion protein with FXa protease site; lanes 6,7, bovine serum albumin (BSA); lanes 8,9, phosphorylase B (Sigma); lanes 10,11, β-galactosidase (Sigma); lanes 12,13, QuantiLum® Recombinant Luciferase.

SPECIFICITY OF PROTEV PROTEASE

ProTEV Protease is manufactured to a high standard of purity and is free from nonspecific activity. Overdigestion of a protein panel with ProTEV Protease results in cleavage of only the control protein containing a ProTEV Protease recognition sequence (Figure 1). Since the ProTEV Protease recognition sequence is uncommon, unintentional cleavage of the protein of interest is rare.

CLEAVAGE CONDITIONS FOR PROTEV PROTEASE

ProTEV Protease is provided with a reaction buffer at pH 7; however, the protease is active over a pH range of 5.5–8.5, allowing it to be used under conditions most amenable to the protein of interest. The greatest activity of ProTEV Protease occurs at 30 °C, but the protease will cleave fusion proteins over a temperature range of 4–34 °C. As with many enzymes, ProTEV Protease cleaves more slowly as the temperature is decreased (Table 1). However, low temperature incubations can be shortened by adding more ProTEV Protease.

Table 1. Time Course of ProTEV Protease Cleavage of GST-MBP at Various Temperatures. Reactions were set up with 20 µg of GST-MBP fusion protein and 1 unit of ProTEV Protease. The reactions were incubated at the indicated temperatures, and aliquots were removed at the indicated times. Samples were analyzed by SDS-PAGE and quantified by densitometry.

Time (minutes)	% Cleavage			
	4 °C	16 °C	22 °C	30 °C
30	32	63	63	76
60	41	77	82	93
120	56	90	92	97
180	64	98	97	98

PROTEV PROTEASE PERFORMANCE VERSUS TRUNCATED TEV PROTEASE

ProTEV Protease is produced in large amounts from *E. coli* as a 50 kDa protein. The other commercially available form of TEV protease is ~28 kDa and contains only the C-terminal catalytic unit of the protease. ProTEV Protease performs equivalently to the truncated version of TEV as tested using a purified fusion protein and fusion protein in a cleared *E. coli* lysate (Figure 2).

PROTEV PROTEASE PURIFICATION FROM CLEAVED PROTEIN

ProTEV Protease has an affinity tag to remove the protease from cleavage reactions. Incubating cleavage reactions with immobilized metal affinity resins like MagneHis™ Ni-Particles (Cat.# V8560) and HisLink™ Protein Purification Resin (Cat.# V8823) quickly and easily removes the protease from the protein of interest (Figure 3, Panel A). If ProTEV Protease is used to remove a polyhistidine or HQ tag from the protein of interest, the small tag would also be removed by the metal affinity resin.

ProTEV Protease can also be used to cleave fusion proteins directly from affinity resins, as shown using glutathione resin (GE Healthcare) and HaloLink™ Magnetic Beads (Figure 3, Panels B and C; Cat.# G9311). The advantage of this technique is that the protein of interest is released into solution while leaving the affinity tag attached to the column, eliminating the need for an additional step to remove the affinity tag from the solution.

CONCLUSIONS

ProTEV Protease is a highly specific protease for cleaving fusion proteins with a TEV recognition site whether the protein is in solution or bound to affinity resins. Since the ProTEV Protease is active over a pH range of 5.5–8.5 and temperature range of 4–34 °C, cleavage conditions can be optimized for the protein of interest. ProTEV Protease performs comparably to commercially available truncated versions of TEV protease. Affinity purification with the HQ tag allows the ProTEV Protease to be easily removed from cleavage reactions.

REFERENCES

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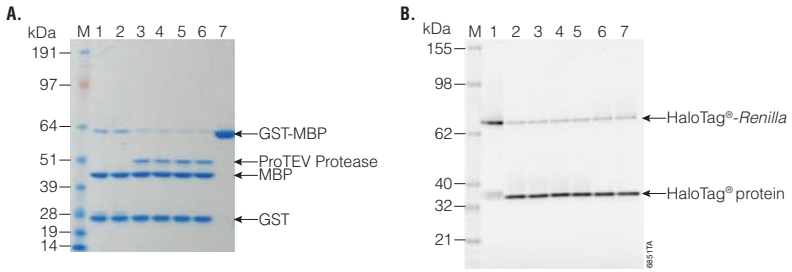

Figure 2. ProTEV Protease performs equivalently to truncated TEV protease. Panel A. GST-MBP fusion protein (20 µg) was digested for 1 hour at 30 °C using 10 units of either ProTEV Protease or truncated TEV protease in 100 µl of the appropriate reaction buffer. Lane M, SeeBlue® Plus2 (Invitrogen); lanes 1,2, truncated TEV protease digest; lanes 3–6, ProTEV Protease digest; lane 7, no protease. **Panel B.** *E. coli* culture expressing HaloTag®-*Renilla* luciferase fusion protein was lysed using FastBreak™ Cell Lysis Reagent (Cat.# V8571). Cleared lysate was labeled with 5 µM HaloTag® TMR Ligand (Cat.# G8251) and cleaved for 1 hour at 30 °C using 10 units of either ProTEV Protease or truncated TEV protease in 100 µl of the appropriate buffer. Lane M, BenchMark™ Fluorescent Protein Standard (Invitrogen); lane 1, no protease; lanes 2–5, ProTEV Protease digest; lanes 6,7, truncated TEV protease digest.

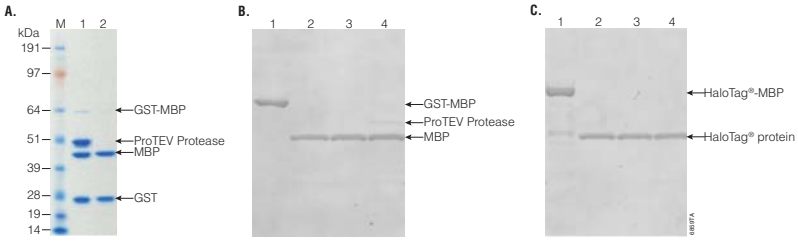

Figure 3. The ProTEV Protease affinity tag confers advantages for protein purification. Panel A. Twenty micrograms of GST-MBP was cleaved for 45 minutes at 30 °C using 10 units of ProTEV Protease in 100 µl of reaction buffer. ProTEV Protease was removed from the reaction by incubation with 25 µl of MagneHis™ Ni-Particle slurry for 30 minutes at room temperature. Lane M, SeeBlue® Plus2 (Invitrogen); lane 1, digest before MagneHis™ Ni-particle incubation; lane 2, digest after MagneHis™ Ni-particle incubation. **Panel B.** Twenty micrograms of GST-MBP fusion protein was bound to a MicroSpin® GST purification module (GE Healthcare) and washed as directed. The resin was then incubated in ProTEV buffer with the indicated amounts of ProTEV Protease overnight at 4 °C on a rotating platform. Lane 1, GST-MBP fusion protein; lane 2, 5 units of ProTEV Protease; lane 3, 10 units of ProTEV Protease; lane 4, 20 units of ProTEV Protease. **Panel C.** Twenty micrograms of HaloTag®-MBP fusion protein was bound to HaloLink™ Magnetic Beads and washed. The resin was then incubated in ProTEV buffer with the indicated amounts of ProTEV Protease overnight at 4 °C on a rotating platform. Lane 1, HaloTag®-MBP fusion protein; lane 2, 5 units of ProTEV Protease; lane 3, 10 units of ProTEV Protease; lane 4, 20 units of ProTEV Protease.

ORDERING INFORMATION

Product	Size	Cat.#
ProTEV Protease	V6051	1,000 units
	V6052	10,000 units

Products may be covered by pending or issued patents or may have certain limitations. Please visit our Web site for more information.