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# TEV Protease, Recombinant*

**Cat. No. 10127-017** **Size: 1,000 units;**
**Lot No.** **Concentration: 10 U/µl**
**Exp. Date:** **Store at -70°C.**

*Licensed under US Patent 5,532,142.

Description:
TEV Protease, Recombinant (rTEV) is a site-specific protease purified from *E. coli* by the affinity tag, poly histidine tag. The protease can be used for the removal of affinity tags from fusion proteins. The seven-amino-acid recognition site for rTEV is Glu-Asn-Leu-Tyr-Phe-Gln-Gly (1-4) with cleavage occurring between Gln and Gly. The optimal temperature for cleavage is 30°C; however, the enzyme can be used at temperatures as low as 4°C (table 1). Following digestion, TEV Protease can be removed from the reaction via the poly histidine tag sequence (by affinity chromatography).

Components:

| | | |
|---|---|---|
| 10127-017 | TEV Protease, Recombinant | Lot No. |
| Y02233 | 20X rTEV Buffer | Lot No. |
| Y00147 | 0.1 M DTT | Lot No. |

**Store rTEV at -70°C for long term or at -20°C for < 6 months.**
Store 20X rTEV Buffer at 4°C, -20°C or -70°C
Store 0.1 M DTT at -20°C or -70°C.

Unit Definition:
One unit of rTEV cleaves ≥95% of 3 µg control substrate in 1 h at 30°C.

Storage Buffer:
50 mM Tris-HCl (pH 7.5)
1 mM EDTA
5 mM DTT
50% (v/v) glycerol
0.1% (w/v) Triton® X-100

Unit Assay Conditions:
50 mM Tris-HCl (pH 8.0)
0.5 mM EDTA
1 mM DTT
3 µg control substrate (5)
1 unit enzyme

20X rTEV Buffer:
1 M Tris-HCl (pH 8.0)
10 mM EDTA

Reaction volume: 30 µl
Incubation: 1 h at 30°C

Doc.Rev. 121200

Quality Control:
This product has passed the following quality control assay: functional absence of any non-specific protease activity.

Cloning of the cleavage site for TEV Protease, Recombinant into vectors:
The cleavage site for rTEV (Glu-Asn-Leu-Tyr-Phe-Gln-Gly) can be introduced into a variety of expression vectors by synthesizing a dsDNA oligo that contains the nucleotides that encode the recognition site amino acids. The recognition site can be cloned directly adjacent to the glutathione-S-transferase domain (pGEX vectors), or maltose-binding domain (pMal vectors). To improve efficiency of rTEV cleavage for poly histidine tag vectors, the addition of a spacer sequence is recommended. The spacer arm sequence (Asp-Tyr-Asp-Ile-Pro-Thr-Thr) is inserted at the carboxyl-terminus of the poly histidine tag, upstream of the rTEV cleavage site.

Recommended Conditions for Cleavage of a Fusion Protein:
A number of variables can be changed to optimize the cleavage of any specific protein. The amount of rTEV, the temperature of the incubation, and the time needed for cleavage may be examined. If the protein of interest is heat-labile, then 4°C incubations are recommended. Reactions at 4°C will require longer incubation times and/or more rTEV.

Example of a time course experiment where the amount of rTEV and temperature are held constant is presented:

1. Add the following components to a microcentrifuge tube:

| | |
|---|---|
| Fusion Protein | 20 µg |
| 20X rTEV Buffer | 7.5 µl |
| 0.1 M DTT | 1.5 µl |
| TEV Protease, Recombinant | 1 µl (10 units) |
| Water | to 150 µl |

2. Incubate at 30°C. Remove 30-µl aliquots at 1, 2, 4 and 6 hours.
3. Add 30 µl 2X SDS-loading buffer [125 mM Tris-HCl (pH 6.8), 4% SDS, 1.4 M 2-mercaptoethanol, 20% (v/v) glycerol, 0.01% bromophenol blue]. Place samples at -20°C until experiment is complete.

4. Boil samples for 5 min and apply 40 µl to an SDS-polyacrylamide gel. Percentage of gel is dependent on the size of protein being analyzed. The percent cleavage of protein is determined by analyzing the amount of uncleaved protein remaining after incubation and the amount of cleaved products that appear.

Varying Parameters for Cleavage

The percent of control substrate hydrolyzed by rTEV at various temperatures was examined (table 1). The optimal temperature for cleavage is 30°C; however, at 21°C and 16°C for one hour, ~80% of control substrate was cleaved. Significant cleavage was observed at 4°C.

Table 1: Cleavage of 3 µg of control substrate with one unit of rTEV at various temperatures.

| Substrate Hydrolyzed | | | | |
|---|---|---|---|---|
| **Time** | **4°C** | **16°C** | **21°C** | **30°C** |
| **0.5 h** | 34 | 58 | 56 | 77 |
| **1 h** | 58 | 80 | 78 | 90 |
| **2 h** | 71 | 99 | 99 | 99 |
| **3 h** | 84 | 99 | 99 | 99 |

More cleaved protein is formed with rTEV by increasing the incubation time (figure 1). However, if time is critical, the addition of more rTEV results in increased hydrolysis (figure 2).

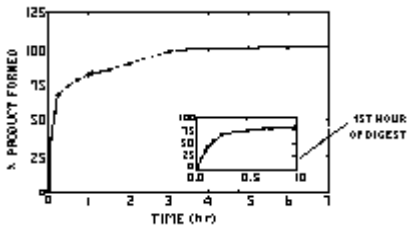


Figure 1: Time course of cleavage reaction. rTEV (0.5 units) incubated with 3 µg control substrate at 30°C.

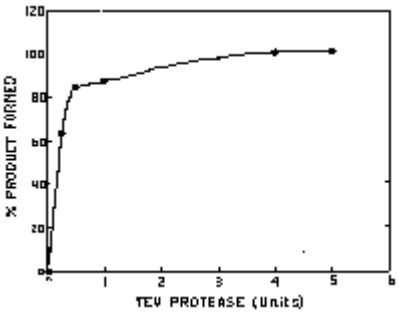


Figure 2: rTEV titration with 10 µg control substrate for 1 h at 30°C.

References:

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2. Carrington, J.C. and Dougherty, W.G. (1988) *Proc. Natl. Acad. Sci. USA 85*, 3391.
3. Dougherty, W.G., *et al*. (1989) *Virology 172*, 302.
4. Dougherty, W.G., and Parks, T.D. (1989) *Virology 172*, 145.
5. Van Hoy, M., *et al*. (1993) *Cell 72*, 587.



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