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TEV Protease

(modified)

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– modified –

(Tobacco Etch Virus)

Cysteine protease from Tobacco Etch Virus for the removal of affinity tags from fusion proteins under target protein friendly conditions. The recombinant protein contains a N-terminal poly-his tag and a C-terminal polyarginine tag for easy removal after cleavage. Genetically engineered for resistance against autolysis, as well as for improved activity and performance.

Cat. No.	Size
E4310-01	1 000 Units
E4310-02	10 000 Units

Unit Definition:
One unit is the amount of enzyme required to cleave >85% of 3 µg control substrate (35 kDa fusion protein) in 1 hour at 30˚C.

Storage Conditions:
Store at -20˚C.
No autolysis.
Protein is stable at least for 9 months.

1 x Reaction Buffer:
25 mM Tris-HCl (pH 8.0), 150 mM NaCl, 14 mM β-mercaptoethanol.

Storage Buffer:
0.4 M NaCl, 50mM Tris-HCl (pH 7.5), 2 mM EDTA, 1mM DTT, 50% (v/v) glycerol.

Quality Control:
Protease is greater than 95% single-band pure without non-specific protease contamination.

References:

1. *Polayes D.A. et al. (1998) Methods in Molecular Medicine Vol.13: 169-183*
2. *Dougherty, W.G. et al. (1989) Virology 172, 302.*
3. *Dougherty, W.G., and Parks, T.D. (1989) Virology 172, 145.*
4. *Dougherty, W.G. et al. (1988) EMBO 7,1281.*
5. *Kapust, R.B., et al. (2002a) Biochem.Biophys. Res. Commun.294: 949-955.*
6. *Nallamsrtty, S. et al. (2004) Protein Expr Purif. 38(1): 108-15.*
7. *Mohanty, A.K. et al. (2003) Protein Expr Purif. 27: 109-114.*

Description:

- → TEV protease is a catalytic part of the Nuclear Inclusion protein "a" (NIa) from tobacco etch virus (TEV).
- → TEV is a cysteine protease that specifically recognizes and cleaves a linear epitope with the general sequence E-X-X-Y-X-Q-(G/S) (where X is any amino acid) (2-5). The cleavage occurs between Q and G/S.The most common sequence is ENLYFQG or ENLYFQS.
- → Resistant to many widely used serine and cysteine protease inhibitors like: PMSF, AEBSF, TLCK, E-64, "Complete" protease inhibitor cocktail (Roche).
- → Robust enzyme active in the wide range of different buffers (with NaCl varied from 0 to 0.4 M and in pH from 4 to 9, enzyme tolerates MES, acetate, phosphate, glycerol and sorbitol).
- → Suitable for tag removal from thermolabile proteins: Active in a broad temperature range from 4˚C to 30˚C (the enzyme is 3 times less active at 4˚C than at 30˚C) (6).
- → Sensitive to some detergents (7).
- → Extremely useful for removing affinity tags from fusion proteins in conditions friendly for target protein.
- → The enzyme is genetically modified towards protection against autolysis (self-cleavage) and increased catalytic activity, as compared to the wild type enzyme.

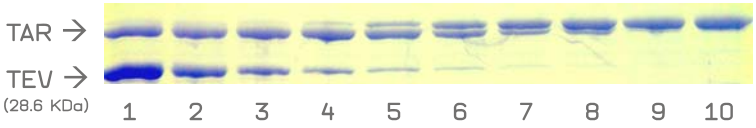

Figure: Determining the amount of TEV Protease for Efficient Cleavage.
The optimal amount of TEV protease for cleavage of a 35 kDa fusion protein is determined by SDS-PAGE analysis. TAR: Target Protein (upper band: fusion protein, lower band: cleaved substrate), TEV: TEV Protease. Lanes 1-9, decreasing amounts of enzyme with fusion protein as a substrate, lane 10, no enzyme control reaction. Cleavage is performed in 1x TEV buffer for one hour at 30˚C. Determination of optimal cleavage temperature (between 4˚C and 30˚C) and time are performed accordingly (1).

Example reaction:

Target fusion protein (example size: 35 kDa)	20 µg
10x TEV buffer	10 µl
TEV Protease	9 U
H_2O	@ 100 µl

- → Incubate for one hour at 30˚C. For heat-labile target fusion proteins, either digest at low temperatures and extend incubation to at least 3 hours or increase the protease concentration.
- → With increasing molecular weight of the target protein, less protease per µg target protein is needed. For example, to cleave 20 µg of a 100 kDa fusion protein,3 U TEV protease are sufficient . In difficult cases (diminished accessibility of cleavage site) it may be necessary to increase the amount of TEV protease.
- → After cleavage reaction, load a sample on a suitable SDS- PAGE and evaluate reaction efficiency by comparing the relation of cleaved products to remains of non-cleaved protein. To optimize the reaction, vary the amount of TEV protease, incubation time and temperature.

Example for the introduction of TEV cleavage sites via PCR:
The following example shows a PCR primer extension for introducing a TEV cleavage site to the 5' end of a PCR amplicon. TEV cleavage site coding positions and their protein translation are displayed in blue color. This example assumes in-frame blunt end cloning (e.g. Sma I). Additionally introduced restriction sites may facilitate further cloning steps but are not essential. This example is taken from (1) and has to be modified to fit the specific experimental design.

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    E   N   L   Y   F   Q   G
   Glu Asn Leu Tyr Phe Gln Gly His Met Val Asp Leu Glu
5'-GAG AAT CTT TAT TTT CAG GGC CAT ATG GTC GAC C-3'-Oligo1
3'-CTC TTA GAA ATA AAA GTC CCG GTA TAC CAG CTG GAG CT 5'-Oligo2
                                    NdeI   Sal1     XhoI
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