TEV Protease

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 of 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.

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., at al. (2002a) Biochem.Biophys. Res. Commun.294: 949-955.*
6. *Nallamsrtty, S. at al. (2004) Protein Expr Purif. 38(1): 108-15.*
7. *Mohanty, A.K. at al. (2003) Protein Expr Purif. 27: 109-114.*

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

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 general sequence E-X-X-Y-X-Q-(G/S)(2-5) (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).
- Active in a broad temperature range from 4 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.

1 x Reaction Buffer:

20 mM Tris-HCl (pH 8.0), 10 mM NaCl, 1 mM $MgCl_2$, 10 mM β-mercaptoethanol.

Storage Buffer:

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

Quality Control:

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

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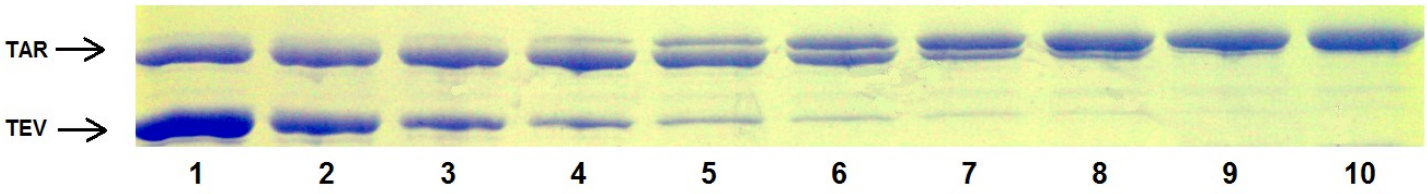

Figure: Determining the amount of TEV Protease for Efficient Cleavage.

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 1 x 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
10 x TEV buffer	10 µl
TEV Protease	9 U
H_2O	to 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 mg 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. SmaI). 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.

	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		SalI		XhoI

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