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AcTEV™ Protease

Cat. No. 12575-015 **Size: 1,000 units**
Cat. No. 12575-023 **Size: 10,000 units**

Description

AcTEV™ Protease is an enhanced form of Tobacco Etch Virus (TEV) protease that is highly site-specific, active, and more stable than native TEV protease (1). AcTEV™ Protease recognizes the seven-amino-acid sequence Glu-Asn-Leu-Tyr-Phe-Gln-Gly (2-5) and cleaves between Gln and Gly with high specificity. The protease is used to cleave affinity tags from fusion proteins. The optimal temperature for cleavage is 30°C; however, the enzyme is active over wide ranges of temperature (see table on page 3) and pH (pH 6.0-8.5). Following digestion, AcTEV™ Protease is easily removed from the cleavage reaction by affinity chromatography using the polyhistidine tag at the N-terminus of the protease. AcTEV™ Protease is purified from *E. coli* by affinity chromatography using the polyhistidine tag.

Components

Item	Composition	Quantity	
		1000 U	**10,000 U**
AcTEV™ Protease (10U/µl)	AcTEV™ Protease in: 50 mM Tris-HCl, pH 7.5 1 mM EDTA 5 mM DTT 50% (v/v) glycerol 0.1% (w/v) Triton® X-100	100 µl	1.0 ml
20X TEV Buffer	1 M Tris-HCl, pH 8.0 10 mM EDTA	1.0 ml	8.0 ml
0.1 M DTT	0.1 M DTT in deionized water	500 µl	2.0 ml

Store AcTEV™ Protease at -20°C or at -80°C for long-term storage.
Store 20X TEV Buffer at 4°C or -20°C and store 0.1 M DTT at -20°C.

Part No. 12575.pps Rev. Date: 07/25/03

Unit Definition

One unit of AcTEV™ cleaves ≥85% of 3 µg control substrate in 1 h at 30°C.

Unit Assay Conditions

The AcTEV™ Protease assay is performed in 1X TEV Buffer (50 mM Tris-HCl, pH 8.0, 0.5 mM EDTA) and 1 mM DTT with 1 unit enzyme and 3 µg control substrate (6) at 30°C for 1 hour in a total volume of 30 µl.

Quality Control

In a quality control assay, this product must demonstrate functional absence of any non-specific protease activity.

Recommended Conditions for Cleavage of a Fusion Protein

Example of a time course experiment with 10 units AcTEV™ Protease is shown below. If the protein of interest is heat-labile, incubate at 4°C with longer incubation times and/or more enzyme (see table on next page).

1. Add the following to a microcentrifuge tube:

Fusion Protein	20 µg
20X TEV Buffer	7.5 µl
0.1 M DTT	1.5 µl
AcTEV™ Protease, (10 units)	1.0 µl
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 sample buffer (125 mM Tris-HCl, pH 6.8; 4% SDS; 1.4 M β-mercaptoethanol; 20% (v/v) glycerol; 0.01% bromophenol blue). Keep samples at -20°C until experiment is complete.
4. Analyze 40 µl of sample by SDS-PAGE using a suitable gel.

The percent protein cleavage is determined by analyzing the amount of cleaved products formed and amount of uncleaved protein remaining after digestion.

After evaluating the initial results, you may optimize the cleavage reaction for your specific protein by optimizing the amount of AcTEV™ Protease, incubation temperature, or reaction time.

Varying Parameters for Cleavage

The percent of 3 µg control substrate hydrolyzed by one unit of AcTEV™ Protease at various temperatures was examined (see table below). More cleaved protein is formed with AcTEV™ by increasing the incubation time. If time is critical, add more AcTEV™ Protease to increase hydrolysis.

	Percentage Substrate Hydrolyzed			
Time	**4°C**	**16°C**	**21°C**	**30°C**
0.5 h	36	62	72	85
1 h	58	85	99	99
2 h	77	99	99	99
3 h	88	99	99	99

Cloning of TEV Cleavage Site into Vectors

You may clone your gene of interest into expression vectors available from Invitrogen that contain an affinity tag and a TEV cleavage site such as Champion™ pET151/D-TOPO (cat. no. K151-01) for *E. coli* expression and pFastBac™ HT (cat. no. 10584-027) for baculovirus expression.

To introduce the TEV cleavage site (Glu-Asn-Leu-Tyr-Phe-Gln-Gly) into an expression vector of choice, design a primer with nucleotides encoding the recognition site amino acids. You may use the free OligoPerfect™ Designer software available on our web site at www.invitrogen.com for primer design. The software conveniently adds a TEV cleavage site into your primer with the click of a button.

Removal of AcTEV™ Protease after Cleavage

The AcTEV™ Protease contains a polyhistidine tag at the N-terminus. After cleavage of the fusion protein, remove AcTEV™ Protease from the cleavage reaction by affinity chromatography on a nickel chelating resin such as ProBond™ Resin (cat. no. K801-01). Dilute cleavage reaction in the binding buffer for ProBond™ and perform binding and elution as described in the ProBond™ Purification manual available at www.invitrogen.com. **The cleaved native protein will be in the flow-through fractions.**

References

1. Nayak, S. *et al*. (2003) *Focus*® in press.
2. Dougherty, W.G., *et al*. (1988) *EMBO 7*, 1281.
3. Carrington, J.C. and Dougherty, W.G. (1988) *Proc. Natl. Acad. Sci. USA 85*, 3391.
4. Dougherty, W.G., *et al*. (1989) *Virology 172*, 302.
5. Dougherty, W.G., and Parks, T.D. (1989) *Virology 172*, 145.
6. Van Hoy, M., *et al*. (1993) *Cell 72*, 587.

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