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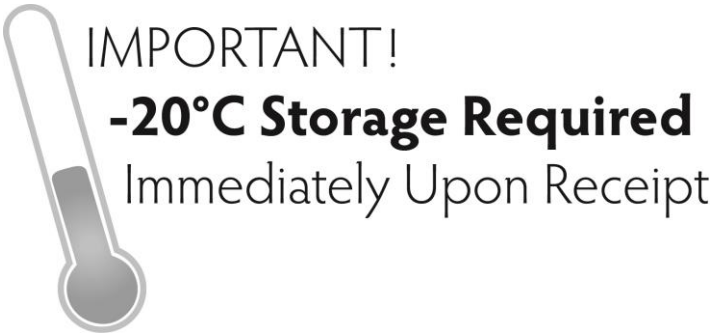
Simplifying Genomics

SelecTEV™ Protease



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Lucigen Technical Support
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Product Guarantee: Lucigen guarantees that this product will perform as specified for one year from the date of shipment. Please avoid using reagents for longer than one year from receipt.

Product Designations

SelecTEV™ Protease is supplied with SelecTEV™ 20X Buffer and 100 mM DTT. The catalog numbers are listed below.

Product	Kit Size	Catalog Number
SelecTEV™ Protease	1,000 Units	30810-1
	5,000 Units	30810-2

Components and Storage

SelecTEV™ Protease and the supplied components must be stored at -20°C.

SelecTEV™ Protease consists of the following components:

Component	Part Number	1,000 Units	5,000 Units
SelecTEV™ Protease (10 U/µL)	F833167	100 µL	100 µL x 5
SelecTEV™ 20X Buffer	F883093-1	1.0 mL	1.0 mL x 5
100 mM DTT	F853091-1	500 µL	500 µL x 5

SelecTEV™ Protease Description

SelecTEV™ Protease is an improved form of Tobacco Etch Virus (TEV) protease that has been engineered to be more specific, active, and stable than the native protease. SelecTEV™ Protease recognizes the seven amino acid sequence Glu-Asn-Leu-Tyr-Phe-Gln∨Gly and closely related sequences. SelecTEV protease cleaves between the Gln and Gly residues. This seven amino acid sequence is rarely found in proteins, making SelecTEV™ Protease an ideal choice for tag removal from fusion proteins.

The optimal temperature for cleavage is 30°C, however the enzyme is active over a wide temperature range (4 – 30°C) and pH (6.0 – 8.5) to accommodate your specific protein. Following digestion, SelecTEV™ Protease is easily removed from the cleavage reaction by affinity chromatography using the polyhistidine tag at the N-terminus of the protease. SelecTEV™ Protease is purified from *E. coli* by affinity chromatography using the polyhistidine tag and is 90% pure when visualized on an SDS-PAGE gel.

Unit Definition

One unit of SelecTEV™ Protease cleaves ≥85% of 3 µg of control substrate in 1 hour at 30°C.

Unit Assay Conditions

The cleavage assay is performed in 1X SelecTEV™ Buffer (50 mM Tris-HCl, pH 8.0, 0.5 mM EDTA) and 1 mM DTT with 10 units enzyme and 30 µg control substrate at 30°C for 1 hour in a total volume of 100 µL.

Recommended Conditions for Cleavage of a Fusion Protein

An example of a cleavage experiment with 10 units of SelecTEV™ Protease is shown below. Optimization of the cleavage conditions may be necessary depending on the protein of interest.

While cleavage occurs optimally in the provided SelecTEV™ Protease Buffer at 30°C, SelecTEV™ Protease is active between 4 – 30°C and pH 6.0 – 8.5.

 **Note:** A precipitate may be observed after thawing of the 20X SelecTEV Buffer. Warm to 37°C and vortex to bring back into solution prior to use.

1. Add the following to a microcentrifuge tube.

Volume, µL	Component	Final Concentration/ Amount
X	Fusion Protein	30 µg
5	SelecTEV™ 20X Buffer	1X
1	DTT, 100mM	1 mM
1	SelecTEV™ Protease, 10 U/ µL	10 U
Y	Water	N/A
100	Total Volume	

2. Incubate the reaction at 30°C for at least 1 hour.

3. Stop the reaction by adding SDS sample buffer (62.5mM Tris-HCl pH 6.8; 2% SDS; 5% β-mercaptoethanol or 0.1M DTT; 25% glycerol; 0.01% Bromophenol Blue) and heating at 95°C for 5 minutes.

4. Analyze 10 µL from step 2 by SDS-PAGE gel.

The percent protein cleavage is determined by analyzing the amount of uncleaved protein remaining after digestion. After evaluating the initial results, the cleavage reaction may be optimized for your specific protein by adjusting the amount of SelecTEV™ Protease, incubation temperature, and/or reaction time.

Removing SelecTEV™ Protease after Substrate Cleavage

SelecTEV™ Protease contains a polyhistidine tag at its N-terminus. After cleavage of the fusion protein, you may remove SelecTEV™ Protease from the cleavage reaction by affinity

chromatography on an immobilized metal affinity chromatography (IMAC) resin such as Ni-NTA (Qiagen), TALON® (Clontech), or His-Select® (Sigma).

Perform the binding and elution as described in the resin manufacturer's protocol. The cleaved native protein will be in the flow-through fractions (as long as the cleaved protein does not contain a histidine tag) and the protease will remain on the resin.

Notes:

- Imidazole remaining in the sample could prevent the polyhistidine tag on SelecTEV from binding to the IMAC resin. Remove or dilute imidazole prior to IMAC purification.
- Many IMAC resins do not tolerate 1 mM DTT. Dilute the sample in column binding buffer or remove DTT prior to purification.

SelecTEV™ Cleavage During Dialysis

The cleavage reaction can be performed during buffer exchange by dialysis. Conditions may be adjusted from those recommended above. In general, use 1 µL SelecTEV (10 U) for every 10 µg of fusion substrate. If dialysis is carried out at 4 °C, an overnight (≥ 16 hours) reaction time is recommended.

SelecTEV™ Protease Cleavage Troubleshooting

There are a variety of reasons that SelecTEV™ Protease may not cleave a particular substrate as expected. Refer to the troubleshooting table below to address your potential issue.

Problem	Probable Cause	Potential Solution
SelecTEV™ Protease is not cleaving my fusion protein.	The cleavage recognition site is not accessible by SelecTEV™ Protease.	Modify the reaction conditions. Variables may include time, temperature, salt concentration, and/or detergent level. See references below (TEV Protease Reference section) for additional information on possible modifications of the reaction conditions.
		Add a linker between the TEV cleavage site and the protein of interest.
	Cleavage reaction is inhibited by components in the sample. Possible inhibitory components include imidazole or detergents.	Remove inhibitory components from sample using dialysis or other methods prior to cleavage.
SelecTEV™ Protease is not captured on the IMAC resin.	Binding of the SelecTEV™ Protease to the affinity resin is inhibited by the presence of imidazole.	Dialyze your protein out of imidazole-containing buffer during SelecTEV cleavage of your fusion protein.

Problem	Probable Cause	Potential Solution
	The capacity of the resin is exceeded.	Test larger amounts of resin.
		Remember to take into consideration any His-tagged fragments released from the fusion protein by SelecTEV cleavage, which will also bind to the resin.
	DTT or EDTA in the sample strips metal from IMAC resin.	Dilute or dialyze the cleavage reaction into buffer without DTT or EDTA before loading sample on column.
SelecTEV™ Protease is demonstrating non-specific cleavage, as evidenced by the presence of more cleavage products than expected.	There may be additional motifs in your fusion protein that SelecTEV Protease recognizes as cleavage sites.	Examine your substrate's amino acid sequence for potential TEV Protease recognition sites. Mutation of these sites may be required.

TEV Protease References

Kapust, R. B., Tözsér, J., Fox, J. D., Anderson, D. E., Cherry, S., Copeland, T. D., and Waugh, D. S. (2001). Tobacco etch virus protease: Mechanism of autolysis and rational design of stable mutants with wild-type catalytic proficiency. Prot. Eng. 14: 993-1000.

Kapust, R. B., Tözsér, J., Copeland, T. D., and Waugh, D. S. (2002a). The P1' specificity of tobacco etch virus protease. Biochem. Biophys. Res. Commun.294: 949-955.

Lundbäck, A.K., Van Den Berg, S, Hebert, H, Berglund H., Eshaghi, S (2008). Exploring the activity of tobacco etch virus protease in detergent solutions. Analytical Biochemistry. 382: 69-71.

Mohanty, A.K, Simmons, C.R, Wiener, M.C. (2002). Inhibition of tobacco etch virus protease activity by detergents. Protein Expression and Purification. 27: 109-114.

Phan, J., Zdanov, A., Evdokimov, A. G., Tropea, J. E., Peters, H. P. K., Kapust, R. B., Li, M., Wlodawer, A., and Waugh, D. S. (2002). Structural basis for the substrate specificity of tobacco etch virus protease. J. Biol. Chem. 277: 50564-50572.

Tozser, J., Tropea, J. E., Cherry, S., Bagossi, P., Copeland, T. D., Wlodawer, A., and Waugh D. S. (2005). Comparison of the substrate specificity of two potyvirus proteases. FEBS J. 272: 514-523.

Van den Berg, S., Lofdahl, P. A., Hard, T., and Berglund, H. (2006). Improved solubility of TEV protease by directed evolution. J. Biotechnol. 121: 291-298.

Online resources:

http://mcl1.ncifcrf.gov/waugh_tech/faq/tev.pdf

http://www.cardiff.ac.uk/biosi/staffinfo/ehrmann/tools/Recognition.htm

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