Thermo SCIENTIFIC

PRODUCT INFORMATION

Thermo Scientific WELQut Protease

#EO0861 500 u (100 µl)

Lot: __ Expiry Date: __

Store at -20°C

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www.thermoscientific.com/onebio

Description

Thermo Scientific WELQut Protease is highly specific, recombinant serine protease of *Staphylococcus aureus.* It recognizes and precisely cleaves recombinant proteins containing an engineered recognition sequence* W- E- L- Q↓X (Trp, Glu, Leu, Gln, X can be any amino acid). The protease cleaves outside the recognition sequence without leaving additional amino acids bound to the target protein. The WELQut™ Protease is active in a broad temperature (4-30°C) and pH (pH 6.5-9.0) range and does not require specific buffers.

In addition, this new protease has several procedural advantages - it is ideal for on-column proteolysis reactions and can be easily removed from reaction mixtures using its built-in His-tag.

* This cleavage sequence is present in expression vector pLATE52 included into the Thermo Scientific aLICator LIC Cloning and Expression Kit 4 (N-terminal His-tag/WQ) available from Thermo Scientific (#K1281).

Features

- Cleaves outside WELQ recognition sequence, without leaving additional amino acids bound to the target protein.
- Highly specific to cognate recognition site, does not generate non-specific product bands, even after long incubation and using excess of protease.
- Easy to remove from the reaction mixture using built-in His-tag.
- Ideal for on-column proteolysis reactions.

Applications

Removal of N-terminal fusion tags from recombinant protein preparations.

Definition of Activity Unit

Each unit is defined as the amount of enzyme required to cleave ≥ 99% of 100 µg of a control protein in 16 h at 20°C.

Enzyme activity is assayed in 100 µl 100 mM Tris-HCl (pH 8.0).

Source

Bacillus subtilis cells with a cloned gene of SplB protease from *Staphylococcus aureus*.

Molecular Weight

22 kDa monomer.

Storage Buffer

Enzyme is supplied in 10 mM Na_2HPO_4; 1.8 mM KH_2PO_4, pH 7.3; 140 mM NaCl; 2.7 mM KCl, 50% glycerol.

Factors that influence WELQut activity

WELQut Protease cleavage efficiency may be affected by various buffers and their chemical components. Pilot digests should be performed with the target protein to evaluate the effect of a given component or buffer.

Influence of various conditions and components on the activity of WELQut Protease is summarized in the following table.

Condition or component	Effect on WELQut protease activity
Tween-80 (0.01-1%)	None
TritonX-100 (0.01-1%)	None
< 2 M Urea	Partially inhibitory
< 0.25 M Guanidine HCl	Partially inhibitory
DTT (5-50 mM)	None
< 100 mM NaCl	None
Imidazole (20-300 mM)	None
< 100 mM Tris-HCl (pH 8)	None
< 100 mM Na_3PO_4 (pH 7.4)	None
< 20 mM K_3PO_4 (pH 7.4)	None

Protocols

A) Optimization of WELQut Protease cleavage

Accessibility of the cleavage site, the adjacent amino acid sequence, and the degree of protein aggregation-all affect the cleavage efficiency. Optimal cleavage conditions must be determined individually for each protein to be cleaved. We recommend testing several enzyme/protein of interest ratios, concentrations, temperatures (4° to 30°C) or incubation time (1 to 16 h) to optimize the efficiency of cleavage.

Optimization of the cleavage conditions should be performed in small-scale reactions using the following protocol as a starting point.

1. Prepare samples:

Component	Amount
Target protein	50 µg
WELQut Protease*	0.5 u, 1 u, 2 u,10 u
1x Reaction Buffer**	Adjust to 50 µl
Total	50 µl

* Enzyme/protein amount ratios are 1:100, 1:50, 1:25, 1:5 (u/µg). For all recommended enzyme/protein ratios except 1:5 (u/µg), use 10x diluted WELQut Protease. Dilute protease in the reaction buffer to the final concentration of 0.5 u/µl.

** We recommend using 10-100mM Tris-HCl, pH 8.0 as the reaction buffer.

2. Incubate at 15–30°C temperature. Take 8 µl aliquot from each reaction after 1, 3, 6, and 16 h (or overnight). Follow standard protocol for preparation of protein samples prior SDS-PAGE analysis.

   Note. For cleavage reactions longer than 16 h, incubation temperatures higher than 20°C are not recommended.

3. Analyze the efficiency of cleavage in each sample by SDS-PAGE.

Once optimal cleavage conditions have been found, the reaction can be scaled up proportionally for cleavage of fusion proteins in solution, in batch or on-column formats.

B) Cleavage of fusion proteins in solution

1. We recommend using 10-100 mM Tris-HCl (pH 8.0), 10-100 mM Na_3PO_4 (pH 7.4) or 10-20 mM K_3PO_4 (pH 7.4) as 1x buffer for the cleavage reaction.
   Note. If target protein will be downstream purified using affinity chromatography, add NaCl and Imidazole to the reaction buffer at 50 mM and 5-20 mM final concentration respectively.
2. Add WELQut Protease to the fusion protein at an optimized protease/protein ratio.
3. Incubate at 15–30°C temperature for the optimal time.
   Note. If target protein is labile or cleavage reaction lasts longer than 16 h, it should be performed in lower temperatures (4-20°C).
4. *Optional: removal of WELQut protease*
   Use the IMAC resins for post-cleavage purification of target protein according to the manufacturer's instructions. In the IMAC column purification format, the protein of interest is eluted with the flow-through, while WELQut protease and the cleaved affinity tag remain bound to the resin.

C) Cleavage of fusion proteins during affinity purification

WELQut Protease can be used for hydrolysis of fusion proteins during affinity purification procedures. The protein of interest will be cleaved while it is still bound to the resin and further eluted while leaving the affinity tag and WELQut Protease, which contains built-in 6x His-tag, bound to the resin. On-column cleavage needs to be optimized for each fusion protein with respect to the amount of protease used and time required for cleavage (*see* protocol A).

1. Evaluate the amount of target protein to be cleaved by SDS-PAGE.
2. Bind the fusion protein to the metal-affinity resin of choice, wash according to the manufacturer's instructions.

(see continued reverse page)

3. Equilibrate the resin/column 2 times with 2-4 resin volume of 50-100 mM Tris-HCl, 50 mM NaCl, 5-20 mM imidazole (pH 8.0) , or buffer with 50-100 mM Na_3PO_4, 50 mM NaCl and 5-20 mM imidazole (pH 7.4) or buffer with 10-20 mM K_3PO_4, 50 mM NaCl, 5-20 mM imidazole (pH 7.4).
4. Prepare mix of WELQut protease and the buffer used for resin/column equilibration in previous step. Use a protease/recombinant protein ratio determined in cleavage optimization experiment. Load the prepared WELQut protease mix on the column.
   Note. If optimal protease/protein ratio was not determined, we recommend using 1:20 or 1:10 u/µg of WELQut to target protein ratio.
5. Incubate at 15–30°C temperature for the optimal time.
   Note. If optimal cleavage reaction time was not determined, we recommend 16 h (or overnight) incubation at 4-20°C.
6. Collect eluate containing the protein of interest. WELQut Protease remains bound to the resin.
7. *Optional*: Additional amount of equilibration buffer (0.5-1.0 settled resin volume) containing 0.2-0.5 M NaCl and 5-20 mM imidazole, may be used to wash the residual amount of protein of interest from the resin/column. Collect this diluted sample separately.
8. *Recommended:* Remove the residual WELQut protease by running the eluate containing protein of interest through the fresh IMAC sorbent. Collect the flow-through.

CERTIFICATE OF ANALYSIS

Purity:≥ 95% confirmed by SDS-PAGE.
Concentration: 5000 U/mL (or 500 u/100ul)
Volume: recorded 100 ul Protease free: (protease activity is not detected in 50 µg of WELQut protease by using QuantiCleave Protease Assay Kit (Thermo Scientific)).
Specific activity: ≥1000 u/mg of WELQut protease.

Quality authorized by:  Jurgita Zilinskiene

Troubleshooting

Problem	Probable cause	Solution
Incomplete cleavage	Suboptimal WELQut Protease to target protein ratio.	Confirm the amount of fusion protein in the digestion. Adjust the amount of WELQut Protease added.
	Insufficient incubation period.	Increase reaction time.
	Recognition site not present or has been altered during the course of cloning.	Verify presence of the WELQut Protease cleavage sequence (Trp-Glu-Leu-Gln-X).
	Recognition site is not accessible.	Reversibly denature protein with non-ionic detergents, denaturants: Tween-80 (0.01-1%), TritonX-100 (0.01-1%)
	Inhibitors present.	Dialyze the fusion protein before cleaving with WELQut Protease.
Incorrect banding pattern (such as multiple bands present) on SDS-PAGE gel following proteolysis with WELQut Protease	Similar secondary recognition sequences in protein of interest.	Adjust reaction conditions to minimize exposure of secondary cleavage sites (such as salt concentration, time, temperature).
	Proteolysis in bacterial host.	Use protease-deficient strains.