MobiTEV Protease1 (HighSpeed), recombinant, His-Tag

Product Information Sheet
 # PR-ETA10010

SUMMARY

shipped on blue ice; store at - 20 °C,
- 80 °C for long term storage

For research use only

Description

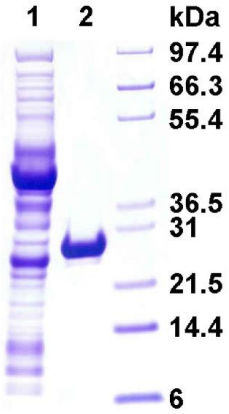
MobiTEV Protease1 (HighSpeed) is used to remove fusion tags - solubility, secretion, detection, and purification tags - and to release the "native" protein of interest. MobiTEV Protease1 (short: MobiTEV1) is an improved version of Tobacco Etch Virus (TEV) protease that is highly site-specific, highly active, and significantly more stable than native TEV Protease, resulting in enhanced long-term activity. MobiTEV1 specifically recognizes a seven amino acid sequence (Glu-Asn-Leu-Tyr-Phe-Gln-Gly, cleaving between Gln and Gly). The MobiTEV1, is fused to a 6xHis-Tag and can be removed from the reaction mixture by Ni-IDA or Ni-NTA chromatography.

Source

The DNA sequence encoding a mutant form of nuclear inclusion protein a (NIa) protease from tobacco etch virus (TEV Protease) was fused to a hexahistidine tag. To avoid proteolytic auto-inactivation, the serine amino acid residue at position 219 was replaced by valine. The chimeric protein was expressed in *E. coli* BL21(DE3) and S219V TEV Protease mutant was purified on a Ni-NTA column.

Molecular Mass

28556 Da (Calculated from amino acid composition).
The affinity purified MobiTEV1 migrates as an approximately 29 kDa protein under non-reducing conditions in SDS-PAGE.



Purity of recombinant MobiTEV Protease1 (HighSpeed). SDS-PAGE analysis.
Soluble cell extract after IPTG induction (lane1), MobiTEV Protease1, affinity purified (lane 2).

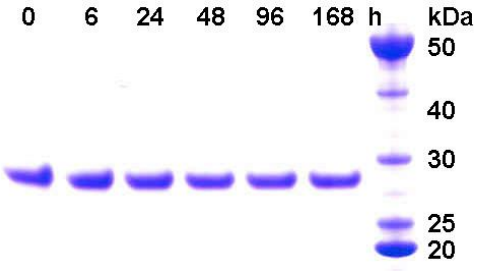
MoBiTec GmbH, Germany Phone: +49 551 70722 0 Fax: +49 551 70722 22 E-Mail: info@mobitec.com www.mobitec.com

Unit Definition

One unit of MobiTEV1 cleaves 3 µg of the control substrate >85% per 1 hour in 30 µl reaction buffer (50 mM Tris/HCl, 0.5 mM EDTA and 1 mM DTT) at pH 8.0 and 30 °C.

Stability and Quality Control

The purified MobiTEV1 did not exhibit any self-cleavage or any non-specific protease activity during the observed time period of 7 days at 4 °C.



Stability assay. 150 U of MobiTEV Protease1 (HighSpeed) were incubated in 1x reaction buffer in a reaction volume of 500 µl. Aliquots (30 µl) were removed at the indicated time points and inactivated with 10 µl of 4x SDS-PAGE loading buffer. The inactivated aliquots were stored at -20 °C until a fraction of each sample was analyzed by SDS-PAGE under reducing conditions (4-12% NuPAGE® Bis-Tris Gel) and Coomassie R-250 staining.

Formulation and Storage

- 1000 U MobiTEV Protease1 (HighSpeed), (5 U/µl): formulated in 50 mM Tris/HCl pH 7.5, 1 mM EDTA, 5 mM DTT, 70 mM imidazole, 50% (v/v) glycerol;
  store at -80 °C; avoid repeated freeze-thaw cycles; stable for 6 months when properly stored.

- 1 ml 20x MobiTEV1 Reaction Buffer,
  1 M Tris/HCl pH 8.0, 10 mM EDTA;
  store at 4 °C

- 500 µl 0.1 M DTT in deionized water;
  store at -20 °C

Standard Protocol

In general 3 U of MobiTEV Protease1 (HighSpeed) are sufficient for cleavage of 100 – 500 µg substrate (fusion protein) in 1x MobiTEV1 Reaction Buffer at 30 °C overnight, with the target protein concentration below 1 mg/ml. As the cleavage efficiency varies due to the flanking sequences around the cleavage site in the target protein, it is recommended to optimize the MobiTEV1-to-target protein ratio using the protocol of following time course experiment:

1. Add the following to a reaction tube:
   25 µg Fusion Protein
   10 µl 20x MobiTEV1 Reaction Buffer
   2 µl 0.1 M DTT
   2,4 µl MobiTEV Protease1 (12 U)
   Add deionized water to 200 µl.

2. Incubate at 30 °C. Remove 50 µl aliquots at 1, 2, 4, and 6 hours.

3. Add 10 µl 6x SDS sample buffer (375 mM Tris/HCl, pH 6.8; 12% SDS; 2 M β-mercaptoethanol; 60% (v/v) glycerol; 0.03% bromophenol blue).

4. Analyze 30 µl of each sample by SDS-PAGE under reducing conditions. The percentage of protein cleavage is determined by comparing the amount of cleaved products formed with the amount of uncleaved protein remaining after digestion.

After evaluating the initial results, the cleavage efficiency of the respective target protein can be optimized with respect to the amount of MobiTEV1, incubation temperature, or reaction time. For heat-labile proteins, we recommend incubation at 4 °C for a minimum of 6 hours. Typically, a cleavage rate of 99 % after 2 hours at an incubation temperature of 16 °C can be obtained, while a comparable cleavage rate was reached in less than 1 hour at 30 °C. Alternatively, you can use twice (6 times) the amount of enzyme when incubation temperature is 16 °C (4 °C) compared to standard temperature of 30 °C. Please note that all these results strongly depend on the target protein of interest.

MobiTEV Protease1 (HighSpeed) is active in the following temperature, time and pH ranges:
Temperature range: 4 °C to 37 °C Time range: 1 to 24 hours pH range: 7 to 9

References

1. Kapust *et al.* (2001) Tobacco etch virus protease: mechanism of autolysis and rational design of stable mutants with wild-type catalytic proficiency. Protein Eng. 14, 993-1000.

2. Pace C. N. *et al.* (1995) How to measure and predict the molar absorption coefficient of a protein. Protein Science 4, 2411-2423.

Order Information, Shipping and Storage

Order#	Product	Amount
PR-ETA10010-02	MobiTEV Protease1 (HighSpeed), recombinant, His-tag	100 U
PR-ETA10010-01	MobiTEV Protease1 (HighSpeed), recombinant, His-tag	1000 U
PR-ETA10010-05	MobiTEV Protease1 (HighSpeed), recombinant, His-tag	10x 1000 U
PR-ETA10010-10	MobiTEV Protease1 (HighSpeed), recombinant, His-tag	100,000 U
Store enzyme at -20 °C, long-term storage -80 °C; Buffer at 4 °C; DTT at -20 °C; avoid repeated freeze-thaw cycles; stable for 6 months when properly stored		

Contact and Support

MoBiTec GmbH ● Lotzestrasse 22a ● D-37083 Goettingen ● Germany

Customer Service – General inquiries & orders
phone: +49 (0)551 707 22 0
fax: +49 (0)551 707 22 22
e-mail: order@mobitec.com

Technical Service – Product information
phone: +49 (0)551 707 22 70
fax: +49 (0)551 707 22 77
e-mail: info@mobitec.com

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