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KRISHZYME™ TEV Protease

Catalog Number: KBENZ71

Protein Description

The KRISHZYME™ TEV Protease (Tobacco Etch Virus nuclear-inclusion-a endopeptidase) is a highly sequence-specific cysteine protease from Tobacco Etch Virus (TEV). It is a member of the PA clan of chymotrypsin-like proteases. TEV Protease is a highly specific cysteine protease that recognizes the amino-acid sequence Glu-Asn-Leu-Tyr-Phe-Gln-(Gly/Ser) and cleaves between the Gln and Gly/Ser residues. Due to its high sequence specificity it is frequently used for the controlled cleavage of fusion proteins in vitro and in vivo.

Source:

Tobacco Etch Virus (TEV)

Expression Host:

E.coli

Purity:

>80% as determined by SDS-PAGE quantitative densitometry by Coomassie Blue Staining.

Endotoxin:

< 0.05 EU/1000 units as determined by the LAL method.

N terminal:

N- terminal Gst Tag

Molecular Mass:

The KRISHZYME™ TEV Protease has a calculated molecular mass of 50 kDa

Concentration:

5 U/ml

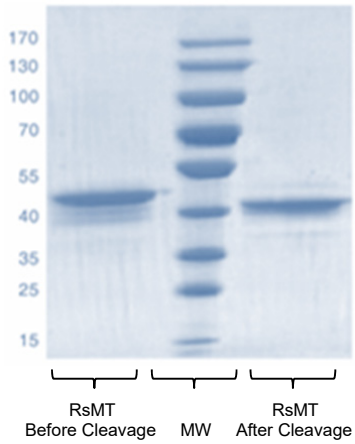
Unit Definition:

One Active Unit is defined as the reaction of 1X TEV Protease Buffer for 3 hours at 30ºC and cleaves >35% of 3 ug substrate

Specific Activity:

17 U/ul

SDS-PAGE:
Fig.1.



KDa Marker 98%

Fig. 1. Purity analysis by SDS-PAGE Detection

Formulation:

KRISHZYME™ TEV Protease is supplied as a liquid containing 25 mM Tris-HCl, 50 mM NaCl, 1 mM EDTA, 50% glycerol. KRISHZYME™ TEV Protease is also supplied as a lyophilized enzyme. Please contact us by email at sales@krishgen.com for the same.

Reconstitution:

Being an enzyme, the concentration may differ from lot to lot. We always recommend referring the accompanying data sheet to view the exact concentration and the recommended dilution schemata.

Centrifuge the vial at 4ºC before opening to recover the entire contents. Please contact us for any concerns or special requirements at +91-22-49198700 | Email: sales@krishgen.com

Storage:

Store it under sterile conditions at -20ºC to -80ºC upon receiving for at least 12 months. It is recommended to aliquot the enzyme into smaller quantities for optimal storage. Avoid repeated freeze-thaw cycles.

Application:

Removal of affinity purification tags such as maltose-binding protein (MBP) or poly-histidine from fusion proteins

Protocol:

Incubation times and enzyme concentrations must be optimized for a particular substrate. Typical reaction conditions are as follows:
1) Combine 15 ug of substrate and H2O (if necessary) to make a 45 ul total reaction volume.
2) Add 5 ul of TEV Protease Reaction Buffer (10X) to make a 50 ul total reaction volume.
3) Add 1 ul of TEV Protease.
4) Incubate at 30°C for 1 hour or at 4°C overnight.

Notes:
1. In order to achieve the best digestion effect, please ensure that the recombinant protein is partially or completely purified protein.
2. If the fusion protein contains the denaturant, the denaturant should be removed to perform the digestion reaction.
3. To remove TEV Protease from the digested system, it is recommend to use a glutathione affinity column.
4. If the fusion protein sample contains >2 M urea, >0.5 M Guanidine hydrochloride, >50 mM imidazole, pH values below 6 or above 9, or cysteine protease inhibitors then it will be necessary to dialyze the fusion protein into TEV protease reaction buffer before TEV Protease cleavage.
5. TEV protease is inhibited by reaction buffers containing >40% Glycerol.
6. Inhibition occurs in the presence of ≥ 5 mM Zn2+, ≥ 1 mM Cu2+ and ≥ 10 mM Co2+.
7. Some substrates may require extended incubation periods (up to three days at either 4°C or 30°C) to achieve complete cleavage. The addition of more TEV Protease after 24 hours may also help achieve complete cleavage of some substrates.

References:

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