Product Information

TEV Protease Kit

Kit Contents

Component	29090-1000U	29090-10000U
29090A: TEV Protease (10 U/uL)	1,000 units	10,000 units
29090B: Control Substrate (1 mg/mL)	10 ug	50 ug
29090C: 10X Reaction Buffer	1 mL	1 mL

Storage and Handling

Store all kit components at -20°C. Product is stable for at least 12 months from date of receipt when stored as recommended.

TEV Protease is supplied at 10 U/uL as a 0.2 um-filtered solution containing 50 mM Tris-HCl, 250 mM NaCl, 1 mM TCEP, 1 mM EDTA, 50% Glycerol, pH 7.5.

Control Substrate is supplied at 1 mg/mL as a 0.2 um-filtered solution containing 50 mM Tris-HCl, 250 mM NaCl, 1 mM TCEP, 1 mM EDTA, 50% Glycerol, pH 7.5.

10X Reaction Buffer is supplied as a 0.2 um-filtered solution containing 500 mM Tris-HCl, 5 mM EDTA, and 10 mM DTT, pH 8.0.

Molecular Weight:

Recombinant 7X His-tag TEV Protease: 28.6 kDa, observed by reducing SDS-PAGE.

Control Substrate: 80.3 kDa (before cleavage); 43.3 kDa and 37 kDa (after TEV cleavage) observed by reducing SDS-PAGE.

Purity (TEV Protease): >95% pure as demonstrated by reducing SDS-PAGE.

Activity (TEV Protease): 10 U/uL.

Unit Definition: One unit of TEV Protease cleaves > 85% of 3 ug of control substrate in 1 hour at 30°C in 1X Reaction Buffer.

Product Description

TEV protease is a highly sequence-specific cysteine protease from Tobacco Etch Virus (TEV). The TEV Protease recognition sequence with the highest catalytic efficiency is the sequence Glu-Asn-Leu-Tyr-Phe-Gln-Ser with optimal cleavage occurring between the Gln and Ser residues. However, the amino acid in the P1' position (amino acid immediately downstream of the cleavage site) can also be Gly, Ala, Met, Cys, or His. The protease is used to cleave affinity tags such as maltose-binding protein (MBP) or poly-histidine from fusion proteins. The optional temperature for cleavage is 30°C but it can also be used at temperatures as low as 4°C.

Biotium's recombinant TEV is expressed from *E. coli* as a single, non-glycosylated polypeptide chain with N-terminal 7X His-tag for easy removal from the reaction using nickel affinity chromatography.

The TEV Kit includes Control Substrate as an optional reaction control. It is a His-tagged fusion protein (MBP-TEV substrate-His6) around 80.3 kDa, observed by reducing SDS-PAGE. When Control Substrate is cleaved with TEV, two bands at 43.3 kDa and 37 kDa are observed under reducing SDS-PAGE (Figure 2).

Recombinant 7X His-tag TEV Protease Sequence

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         10          20          30          40
GHHHHHHHGE SLFKGPRDYN PISSTICHLT NESDGHTTSL
         50          60          70          80
YGIGFGPFII TNKHLFRRNN GTLLVQSLHG VFKVKNTTTL
         90         100         110         120
QQHLIDGRDM IIIRMPKDFP PFPQKLKFRE PQREERICLV
        130         140         150         160
TTNFQTKSMS SMVSDTSCTF PSSDGIFWKH WIQTKDGQCG
        170         180         190         200
SPLVSTRDGF IVGIHSASNF TNTNNYFTSV PKNFMELLTN
        210         220         230         240
QEAQQWVSGW RLNADSVLWG GHKVFMVKPE EPFQPVKEAT

QLMNRRRRR
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Figure 1. Amino acid sequence for recombinant 7X His-tag TEV Protease.

Experimental Protocol

Optimal incubation times and enzyme concentrations may vary between substrates and should be determined empirically. As a starting point, we recommend using 1 unit of TEV protease per 1.5 ug substrate. Reactions may be scaled up linearly. Typical reaction conditions for cleaving Control Substrate in a 50 uL reaction are provided below:

Table 1. Control Reaction Setup

Component	Stock concentration	Volume	Final concentration (50 uL reaction)
Water	N/A	29 uL	N/A
Reaction Buffer	10X	5 uL	1X
Control Substrate	1 mg/mL	15 uL	0.3 mg/mL (15 ug total)
TEV Protease	10 U/uL	1 uL	0.2 U/uL (10 U total)

1. Combine the reaction components in the order shown in Table 1.
2. Incubate at 30°C for 1 hour or at 4°C overnight.
3. Optional: Isolate the cleaved substrate by running the sample reaction over a nickel affinity column and collect the flow through. The Tev Protease and Control Substrate with His tag will remain bound to the column. The MBP tag will be in the flow through. Alternatively, the reaction can be analyzed by SDS-PAGE without column clean-up.
4. Reaction efficiency of TEV protease can be assessed by running a reducing SDS-PAGE of Control Substrate in the presence and absence of TEV protease. The cleaved Control Substrate will produce bands at 37 kDa and 43.3 kDa. Uncleaved Control Substrate will run at 80.3 kDa. If TEV is not removed by nickel column, it will be present on the gel at 28.6 kDa (Figure 2).

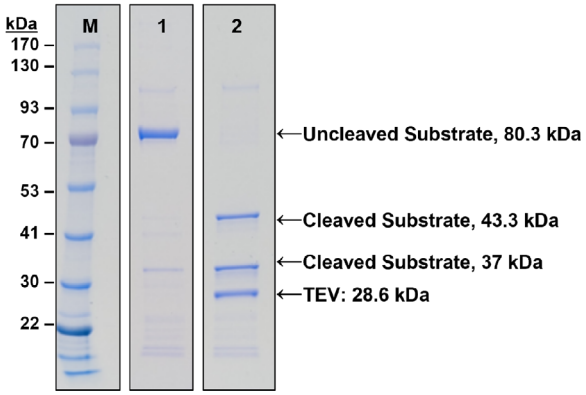

Figure 2. Reducing SDS-PAGE and One-Step Blue® staining of Control Substrate (3 ug) in the absence (Lane 1) or presence (Lane 2) of TEV Protease. Samples were incubated at 30°C for 1 hour prior to gel analysis. Minor band around 100 kDa may be detectable in uncleaved and cleaved Control Substrate samples.

Related Products

Cat. No.	Product
29088	Annexin V (His Tag)
20228... 20360	Monoclonal Mouse Anti-6X His
20014... 20835	Donkey Anti-Mouse IgG (H+L), Highly Cross-Adsorbed (Min X Rat)
20301... 20903	Goat Anti-Mouse IgG (H+L), Highly Cross-Adsorbed (Min X Rat)
21003-1L	One-Step Blue® Protein Gel Stain
21004	One-Step Lumitein™ Protein Gel Stain
21005	One-Step Lumitein™ UV Protein Gel Stain
21530	Peacock™ Prestained Protein Marker
21531	Peacock™ Plus Prestained Protein Marker
40136	4X Protein Loading Buffer with Orange Tracking Dye
23013	TrueBlack® WB Blocking Buffer Kit
22013	Bovine Serum Albumin Fraction V
22012	Dry Milk Powder
22011	Fish Gelatin Powder
22002	Tween® 20
41024-4L	Water, Ultrapure Molecular Biology Grade

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