Recombinant TEV Protease (rTEVase)
Catalog #MB9028
1 mg

Description

TEV (Tobacco Etch Virus) Protease is a highly specific cysteine protease that recognizes a linear epitope of the general form E-X_{aa}-X_{aa}-Y -X_{aa}-Q-(G/S) and cleaves between Q and G/S. The most commonly used sequence is ENLYFQG. Its specificity is more stringent than that of factor Xa, thrombin or enterokinase. The TEV protease is a useful reagent for purification tag removal during recombinant protein purification. ScienCell's engineered TEV protease (rTEVase) has higher stability compared to wild-type TEV protease and is fused with a N-terminal His-tag to facilitate its removal from the digested protein sample.

Kit Components

Cat #	Component	Composition	Quantity	Storage
MB9028a	TEV Protease 1 mg/mL	TEV Protease in: 20 mM Tris-HCl, pH 7.5 50 mM NaCl 5 mM 2-Mercaptoethanol 50% (v/v) glycerol	1 mL	-80 °C
MB9028b	20X TEV Protease Buffer	800 mM Tris-HCl, pH 8.0 4 mM EDTA 20 mM DTT	1.5 mL x2	-20 °C

Additional Materials Required (Not Provided):

6x Laemmli sample buffer

Specifications

Source:	*Escherichia coli*
Molecular Weight:	28 kDa
Biological Activity:	One unit of TEV protease is defined as the amount of enzyme needed to cleave 85% of 3 µg of control protein with ENLYFQG cleavage site at 30 °C for 1 hour. ≥10,000 U/mg TEV protease

Quality control

TEV Protease has greater than 90% single-band purity by SDS-PAGE (Figure 1). Representative gel picture shows specific cleavage activity (Figure 2). Non-specific protease activity is undetectable.

Product Use

rTEVase is for research use only. It is not approved for human or animal use, or for application in clinical or *in vitro* diagnostic procedures.

Shipping and Storage

Dry Ice. Upon receipt, store TEV protease (MB9028a) at -80ºC and buffer (MB9028b) at -20ºC. Aliquot after the first thawing. For convenience, the TEV protease (MB9028a) may be stored at -20ºC for up to 3 months. Please avoid repeated freeze/thaw cycles. Stable for 24 months when stored properly.

Procedure

TEV protease is active over a wide range of temperatures (2-37 ºC) and pH ranges (4-9). The activity could reduce to ~50% activity when 0.5 M NaCl or 200 mM imidazole is present in reaction buffer. Researchers will need to optimize their specific reaction conditions. As an initial suggestion, an example of a time course experiment with TEV protease is provided.

1. Prepare cleavage reaction as shown in Table 1.

Table 1. Suggested Reaction System

Component	Volume	Final concentration
Fusion protein with TEV cleavage site	variable	100 µg
TEV protease	2 µL	2 µg
20× TEV Protease Buffer	5 µL	1 x
ddH_2O	variable	-
Total volume	100 µL	-

2. Mix and incubate at 4ºC, 16ºC and 30ºC.Time will vary depending on temperature as indicated in Table 2. Remove 10 µl aliquots at each time point as shown in Table 2. Mix the removed aliquots with 10 µl ddH_2O and 4 µl 6x Laemmli sample buffer (NOT included) to stop reactions.

Table 2. Recommended sampling times at different temperatures

Temperature	Times
4 ºC	16 and 32 hours
16 ºC	2, 4, 8 and 16 hours
30 ºC	1, 2, 4 and 8 hours

3. Analyze 10 µl of samples by SDS-PAGE gel.

Determine the percent of protein cleavage by analyzing the amount of cleaved products formed and amount of uncleaved protein remaining after digestion. After evaluating the initial results, you may optimize the cleavage reaction for your specific protein by optimizing the amount of TEV Protease, incubation temperature, or reaction time.

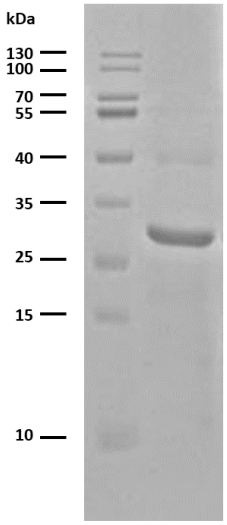

Figure 1. Coomassie staining of TEV Protease. Recombinant TEV Protease (4 µg) resolved on a 12% SDS-PAGE gel under reducing conditions and stained with Coomassie Brilliant Blue.

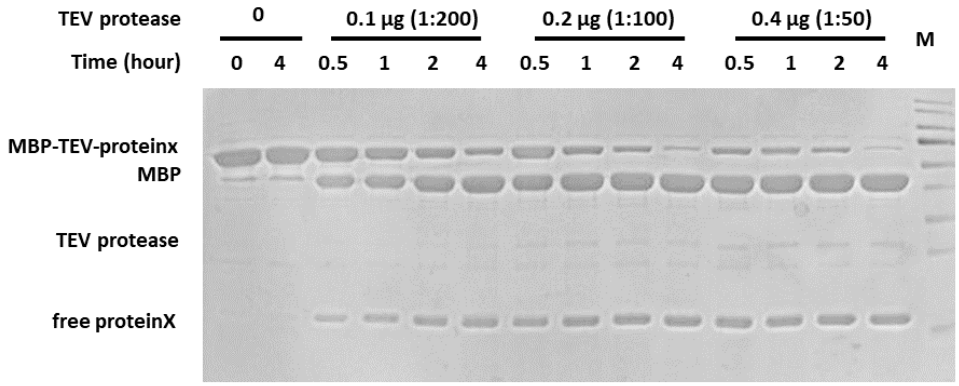

Figure 2. Representative gel picture of TEV protease activity test. A 20 µg of target MBP-TEV-protein was incubated with various amount of TEV protease in a buffer containing 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 50 mM imidazole and 10 mM 2-Mercaptoethanol at 30 ℃. Samples were taken at indicated time points and quenched by Laemmli sample buffer. Sample was resolved on a 15% SDS-PAGE gel under reducing conditions and stained with Coomassie Brilliant Blue.