

## TEV Protease, Recombinant

Catalog No. SG3030-0101

Size: 2500 units

### Description

TEV Protease, Recombinant (rTEV) is a site-specific protease, which can be used for removal of affinity tags from fusion proteins. rTEV protease recognizes the seven-amino-acid sequence Glu-Asn-Leu-Tyr-Phe-Gln-Gly and cleaved between Gln and Gly with high specificity. The optimal temperature for cleavage is 30°C; however, the enzyme can be used at temperatures as low as 4°C (See table on Page 2). Following digestion, rTEV Protease is easily removed from the cleavage reaction by affinity chromatography using the 6xHis tag at the N-terminus of the protease. rTEV Protease is purified from *E. coli* by affinity chromatography using the 6xHis tag.

### Components

Cat. No.	Item	Composition	Quantity
SG3030-0101	rTEV Protease	Lyophilized powder	2,500 units
SG3030-0102	1X rTEV Reconstitution Buffer	50% glycerol	1 ml
SG3030-0103	20X rTEV Reaction Buffer	50 mM Tris-HCl, pH 8.0, 0.5 mM EDTA	1 ml
SG3030-0104	100 mM DTT	100mM DTT in deionized water	1 ml
SG3030-0105	Cleavage Control Protein	Cleavage Control Protein in buffer	500 ug

Store rTEV Protease lyophilized powder, 1X rTEV reconstitution Buffer, 20X rTEV reaction Buffer and 100mM DTT at 4°C, store Cleavage Control Protein at -20°C.

This product is distributed for laboratory research only. CAUTION: Not for diagnostic use. The Safety and efficacy of this product in diagnostic or other clinical uses has not been established.

### Molecular Weight

27kDa

### Unit Definition

One unit of rTEV cleaves ≥85% of 3µg control substrate in 1 h at 30°C.

### Reconstitution

rTEV is Reconstituted with 1 ml rTEV Reconstitution Buffer and then stored at -80°C for long term or at -20°C for < 6 months storage.

### Unit Assay Conditions

The rTEV Protease assay is performed in 1X TEV Reaction Buffer (50mM Tris-HCl, pH 8.0, 0.5mM EDTA) and 1mM DTT with 1 unit enzyme and 3µg substrate at 30°C for 1 hour.

### Quality Control

In a quality control assay, this product must demonstrate functional absence of any non-specific protease activity.

### Recommended Conditions for Cleavage of a Fusion Protein

1. Dissolve the rTEV lyophilized powder using 1ml 1x rTEV Reconstitution Buffer.
2. Add the following to a microcentrifuge tube:

Fusion protein	30ug
rTEV Protease	10U
20x rTEV Reaction Buffer	3ul
0.1M DTT	0.6ul
Add water to	60ul
3. Incubate at 4°C and 30°C. Remove 10ul aliquots at 0.5h , 1h , 2h and 3h
4. Add 10ul 2X SDS loading Buffer, heating at 100°C for 10min.
5. Analyze 8ul of sample by SDS-PAGE using a 12% gel

The percent protein cleavage is determined 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 rTEV Protease, incubation temperature, or reaction time.

### Varying Parameters for Cleavage

The percent of 3ug control substrate hydrolyzed by one unit of rTEV Protease at various temperatures was examined (see table below). More cleaved protein is formed with rTEV by increasing the incubation time. If time is critical, add more rTEV Protease to increase hydrolysis.

Percentage Substrate Hydrolyzed		
Time	4°C	30°C
0.5h	33.6	77.4
1h	45.3	94.1
2h	56.7	95.5
3h	66.5	96