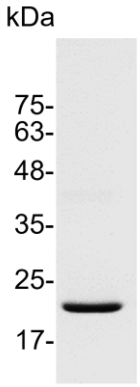
PRODUCT INFORMATION

UniHRV 3C Protease

v. 230201

Catalog number	C09003-bulk / C09003-500U UniHRV 3C Protease C09002-B-bulk / C09002-B-1 10X HRV 3C Cleavage Buffer
Package	Cat. / Name / Amount C09003-500U: UniHRV 3C Protease 500U; Cleavage Validated Protein 20 µg; 10X HRV 3C Cleavage Buffer 10 mL
Description	UniHRV 3C Protease is a recombinant form of the 3C protease derived from human rhinovirus 14 expressed in E. coli (specific activity 1800-2000 U/mg). This product is a highly purified recombinant 6XHis-fusion protein and requires neither metal nor cofactors for activity. UniHRV 3C Protease recognizes the cleavage site: Leu-Glu-Val-Leu-Phe-Gln↓Gly-Pro (LEVLFQ↓GP). UniHRV 3C Protease demonstrate excellent cleavage efficiency in a variety of fusion proteins.
Source	*Escherichia coli*
Endotoxin level	<1 EU per 1 µg of the protein by the LAL method.
Activity	One unit of HRV 3C Protease is defined as the amount of enzyme needed to digest 1 nM of the pNA-peptide substrate per 10 min at RT or per hour at 0 °C in the reaction buffer.
Purity	>98% as determined by SDS-PAGE analysis.
Formulation	UniHRV 3C Protease was lyophilized from a solution containing 50 mM Tris, 150 mM NaCl, 1 mM EDTA, 1 mM DTT, 0.04% Tween20, 8% trehalose, 8% mannitol. 10X HRV 3C Cleavage Buffer: 1.5 M NaCl, 0.5 M Tris-HCl, pH 7.5
Reconstitution	It is recommended to reconstitute the lyophilized protein in sterile H_2O and incubate the stock solution for at least 20 min to ensure sufficient re-dissolved.
Storage	Lyophilized protein should be stored at -20°C. Upon reconstitution, protein aliquots should be stored at -20°C. HRV 3C Protease Cleavage Buffer should be stored at -20°C or 4°C.



SDS-PAGE analysis of UniHRV 3C Protease

Manuel

The following protocol is an example of a simple optimization experiment designed to estimate the appropriate ratio of enzyme:target protein. This example represents UniHRV 3C Protease:target protein ratios (U/μg) of 1:10, 1:25 and 1:50.

Component	Volume (μL)
UniHRV 3C Protease (1 U/μL)	1
Validated Protein (10, 25, and 50 μg each)	X
10X HRV 3C Cleavage Buffer	5
H_2O	44-X
Total volume	50

- Incubate the reaction mixture at 4°C for 16 hours or overnight.
- Determine cleavage level of the samples by SDS-PAGE analysis.
- UniHRV 3C protease: target protein ratio of 1:5~1: 200 (U/μg) is used for most fusion protein cleavage.
- If shorter incubation time is required, more amount of UniHRV 3C protease or higher temperature (RT) can be implemented. Reaction can be performed at 4°C-37°C. 4°C is recommended as the starting standard.

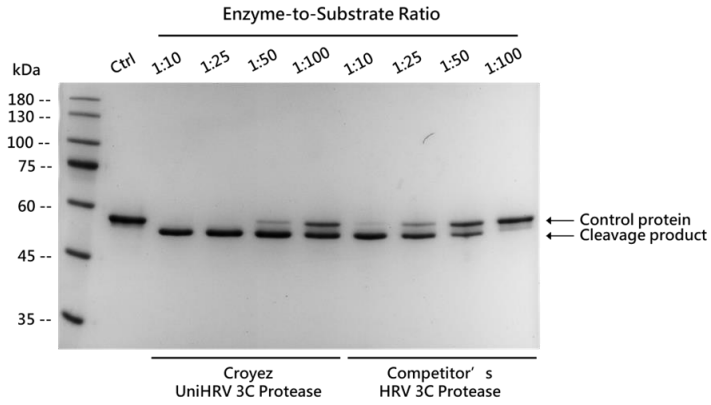


Fig. The validated protein was cleaved by UniHRV 3C protease and Compititor's HRV 3C Protase at 4℃ for 16 h.

Notes (Important)	• Cleavage efficiency may differ based on structure and properties of each target protein, we recommend testing several enzyme-to-substrate ratios, temperatures, and incubation times. • HRV 3C Protease reactions can be performed in a buffer which is optimal for the target protein. Reducing reagents (e.g., DTT) or salts (e.g., NaCl) can be added for cleavage efficiency evaluation. • Usually, 1 U UniHRV 3C Protease can cleavage >95% of target protein (from 50 to 400 µg) at 4˚C for 16h. Even different lot of target proteins might result in different cleavage efficiency. The validated protein (50 µg) provide in this product can be stably cleavage >50% when using 1 U of UniHRV 3C Protease.

For Research Use Only. Not for use in diagnostic and therapeutic procedures.