

Recombinant HRV 3C Protease

Catalogue Number: S3CP01



Sino Biological Inc.
Biological Solution Specialist

Content

2000 U	Recombinant HRV 3C Protease (lyophilized from 50mM Tris, 150mM NaCl, 1mM EDTA, 1mM DTT, 0.04% Tween20, 8% trehalose, 8% mannitol)
100 µg	Cleavage Control Protein (lyophilized from sterile PBS, pH 7.4)
5 ml	10X HRV 3C Cleavage Buffer (1.5 M NaCl, 0.5 M Tris-HCl, pH 7.5)

Description

HRV 3C Protease encoded by human rhinovirus 14 is a highly purified recombinant cysteine protease with a His-tag. Recombinant HRV 3C Protease is a ~20KDa single-chain protein containing approximately 189 amino acids with calculated pI 8.46. HRV 3C protease folds into two anti-parallel six-stranded β -barrels and the site cleft is located at the junction of the two β -barrels domains. The enzyme requires neither metal nor cofactors for activity. It has been demonstrated that the enzyme exhibits highest activity around neutral pH at temperature ranging from 22 to 37 °C, even retaining robust activity at 4 °C. Thus, cleavage can be performed at low temperature to enhance the stability of the target protein. The catalytic activity is insensitive to organic solvents (up to 10%); however, it can be strongly stimulated by high concentration of anions such as sulfate.

Specificity

The enzyme recognizes the cleavage site: Leu-Glu-Val-Leu-Phe-Gln-↓-Gly-Pro

Molecular Weight

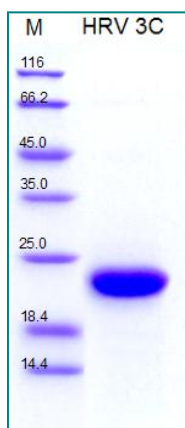
~22KDa on SDS-PAGE

Storage

Store HRV 3C Protease at -20 °C. Store HRV 3C Cleavage Control Protein and Protease Cleavage Buffer at -20 °C or 4 °C.

Purity

98% by SDS-PAGE.



Quality Control

The purity of each lot is determined by SDS-PAGE. And the activity is ensured by cleavage test with a recombinant fusion protein for each lot. The solution of HRV 3C protease is filtered through 0.22µm sterile filter before package.

Application

The high specificity of HRV 3C protease makes it an ideal tool for cleaving fusion proteins at definite cleavage sites. The fusion protein can be purified and cleaved by HRV 3C to obtain the target protein. The recombinant HRV 3C protease is easily removed by IMAC Ni-charged resin.

Activity definition

One unit of HRV 3C Protease is defined as the amount of enzyme that will cleave >95% of 100µg HRV 3C cleavage control protein in 150mM NaCl, 50mM Tris-HCl pH 7.5, at 4 °C for 16h.

User Protocol

Starting conditions

Temperature: 4 °C
Incubation time: 16 hours or overnight
Enzyme amount: 1:25~1: 100 (U/µg)
Empirically, a HRV 3C protease: target protein ratio of 1:25~1: 100 (U/µg) at 4 °C for 16 hours is applicable for most fusion protein cleavage.

Small scale optimization

Due to various properties of fusion proteins, the ratio of HRV 3C protease: target protein, temperature, incubation time is recommended to be optimized for practical application.

The following protocol is a simple example to estimate the appropriate amount of the enzyme.

1. Combine 100µg fusion protein, 10µl 10X HRV 3C protease Cleavage Buffer, HRV 3C protease of different volumes and sterile water to make a 100µl total reaction volume. A control sample without HRV 3C protease should be included to detect a possible unspecific cleavage either by autolysis or by proteolytic contaminations of the fusion protein.

Component	Volume
enzyme vol.(µl)	0, 0.5, 1, 2
100µg control protein	X
10X Cleavage buffer	10
H ₂ O	Y
total volume(µl)	100

2. Incubate the reaction mixture at 4 °C for 16 hours or overnight.
3. Take out 20µl sample and add 20µl 2XSDS-PAGE loading buffer for each treatment and store at -20 °C until SDS-PAGE analysis. If practical, take out aliquots at different time spots to optimize the incubation time.
4. Determine and compare the extent of cleavage of the samples by SDS-PAGE analysis.

If shorter incubation time is required, more amount of HRV 3C protease or higher temperature (RT) can be implemented.

Scale up

When the cleavage conditions are optimized at a small scale, scale up the cleavage proportionally according to specific application requirement.

If IMAC Ni-charged resin is used after cleavage to remove the HRV 3C protease, the buffer of target protein should be exchanged into suitable buffers without EDTA or imidazole. Buffer exchange can be carried out by desalting or dialysis.

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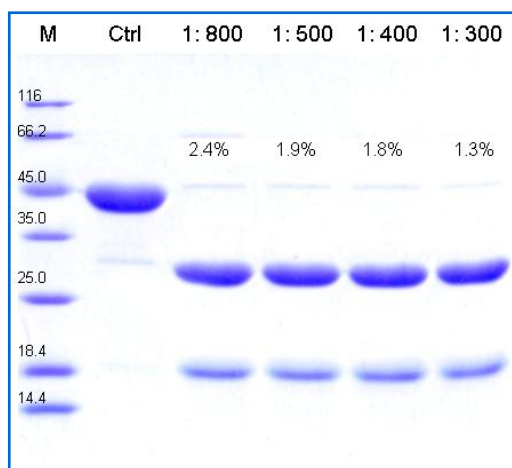


Fig. Optimization of HRV 3C protease cleavage at 4°C for 16 h. A MBP-fusion protein at 1.17mg/ml was cleaved at various ratios of HRV 3C protease to the fusion protein.

Impact of factors on HRV 3C protease activity

Factor	Reagent	Concentration	Relative Activity (%)
Salt	NaCl	0.8M	150
		0.2M	110
		2.5-3M	200
	ZnCl ₂	0.2mM	0
	Na ₂ SO ₄	0.8M	1570~7200
Protease inhibitor	EDTA	50mM	100
	EGTA	50mM	100
	Egg White cystatin	8μm	100
	E-64	100μm	100
	Iodoacetamide	1.0±0.1mM	50
	Pepstatin	20μm	100
	Aprotinin	15μm	100
	Benzamidine	50mM	100
Denaturant	Urea	3M	0
		2M	0
		1M	40
	Guanadine	3M	0
		2M	0
1M	0		
Reductant	DTT	1mM	100
Detergent	Triton X-100	0.10%	>100
		1%	100
	Tween 20	0.10%	>100
		1%	100
	Nonidet P-40	0.10%	>100
		1%	100
Anion(Na salt)	F ⁻	0.2M	250
		0.4M	470
	Cl ⁻	0.2M	110
		0.4M	130

Br ⁻	0.8M	150	
	0.2M	90	
	0.4M	85	
I ⁻	0.8M	81	
	0.2M	81	
	0.4M	63	
CH ₃ CO ₂ ⁻	0.8M	54	
	0.2M	150	
	0.4M	181	
SO ₃ ²⁻	0.8M	338	
	0.2M	122	
	0.4M	220	
SO ₄ ²⁻	0.8M	365	
	0.2M	252	
	0.4M	680	
	0.8M	1570	
	1M	2200	
Co-solvent	Acetonitrile	10%	48
	DMSO	10%	74
	Isopropanol	10%	74
	Methanol	10%	91
	Glycerol	10%	114
	Ethylene glycol	10%	95
	PEG-3400	10%	90
	Sorbitol	10%	120
Sucrose	10%	112	
Elute buffer	Imidazole	20~250mM	100

References

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- [2] Q.May Wang and Shu-Hui Chen. (2007) Human Rhinovirus 3C Protease as a Potential Target for the Development of Antiviral Agents. *Current Protein and Peptide Science*, 8: 18-27
- [3] Matthews, D.A., Smith, W.A., Ferre, R.A., Codon, B., Budahazi, G., Sisson, W., Villafranca, J.E., Janson, C.A., McElroy, H.E., Gribkov, C.L. and Worland S. (1994) Structure of Human Rhinovirus 3C Protease Reveals a Trypsin-like Polypeptide Fold, RNA-Binding Site, and Means for Cleaving Precursor Polyprotein. *Cell*, 77, 761-771.
- [4] Q. May Wang, Robert B. Johnson. (2001) Activation of Human Rhinovirus-14 3C Protease. *Virology* 280, 80-86

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