Recombinant HRV 3C Protease
Catalogue Number: S3CP01

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.

Reconstitution
Resuspend the enzyme powder with sterile water. Keep reconstituted enzyme at -20°C in aliquots.

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.

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>enzyme vol.(μl)</td>
<td>0, 0.5, 1, 2</td>
</tr>
<tr>
<td>100μg control protein</td>
<td>X</td>
</tr>
<tr>
<td>10X Cleavage buffer</td>
<td>10</td>
</tr>
<tr>
<td>H2O</td>
<td>Y</td>
</tr>
<tr>
<td>Total volume(μl)</td>
<td>100</td>
</tr>
</tbody>
</table>

2. Incubate the reaction mixture at 4°C for 16 hours or overnight.

3. Take our 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.

**Enzyme-to Control Protein Ratio**

![Image of Enzyme-to Control Protein Ratio](image_url)

**Fig.** The control protein was cleaved by HRV 3C protease at 4°C for 16 h.

**Impact of factors on HRV 3C protease activity**

<table>
<thead>
<tr>
<th>Factor</th>
<th>Reagent</th>
<th>Concentration</th>
<th>Relative Activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salt</td>
<td>NaCl</td>
<td>0.8M</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.2M</td>
<td>110</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.5-3M</td>
<td>200</td>
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<tr>
<td></td>
<td>ZnCl2</td>
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<td></td>
<td>Na2SO4</td>
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<td>1570-7200</td>
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<td>Protease inhibitor</td>
<td>EDTA</td>
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<tr>
<td></td>
<td>EGTA</td>
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<tr>
<td></td>
<td>Egg White cystatin</td>
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</tr>
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<td></td>
<td>E-64</td>
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<td>100</td>
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<tr>
<td></td>
<td>Iodoacetamide</td>
<td>1.0±0.1mM</td>
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<tr>
<td></td>
<td>Pepstatin</td>
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<tr>
<td></td>
<td>Aprotinin</td>
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<tr>
<td></td>
<td>Benzamidine</td>
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<td></td>
<td>Leupeptin</td>
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<tr>
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<td>0</td>
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<td>Detergent</td>
<td>Triton X-100</td>
<td>0.10%</td>
<td>&gt;100</td>
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**References**


