Recombinant HRV-3C Protease Cleavage Enzyme (with GST-tag)

Contents

YCP1208	2.5 KU or 50 KU	Recombinant HRV 3C Protease Lyophilized powder
YCZ1001	100 µg	Control Protein containing 3C cleavage site.
YCZ1002	10 ml or 100 ml	10X HRV 3C Cleavage Buffer (500mM Tris-HCl, pH-7.0, 1.5 M NaCl, 10 mM EDTA, 10 mM DTT)

Description

Recombinant GST-HRV 3C Protease is a recombinant form of human rhinovirus (HRV) type 14 3C protease (22KDa on SDS-PAGE) produced in Escherichia coli cells. It can be used to cleave recombinant proteins specifically at the following site: Leu-Glu-Val-Leu-Phe-Gln-↓-Gly-Pro, making the enzyme an ideal tool for releasing purification tags from fusion proteins.

Unit Definition

One unit cleaves 100 µg control fusion protein to >95% completeness in 1X cleavage buffer at 4oC for 16 h.

Usually 1 unit is equal to 0.8 µg of protein.

Formulation

Lyophilized after filtrated through 0.22 µm filter in a solution of 50 mM Tris, 150 mM NaCl, 1 mM EDTA, 0.05% Tween20.

Reconstitution

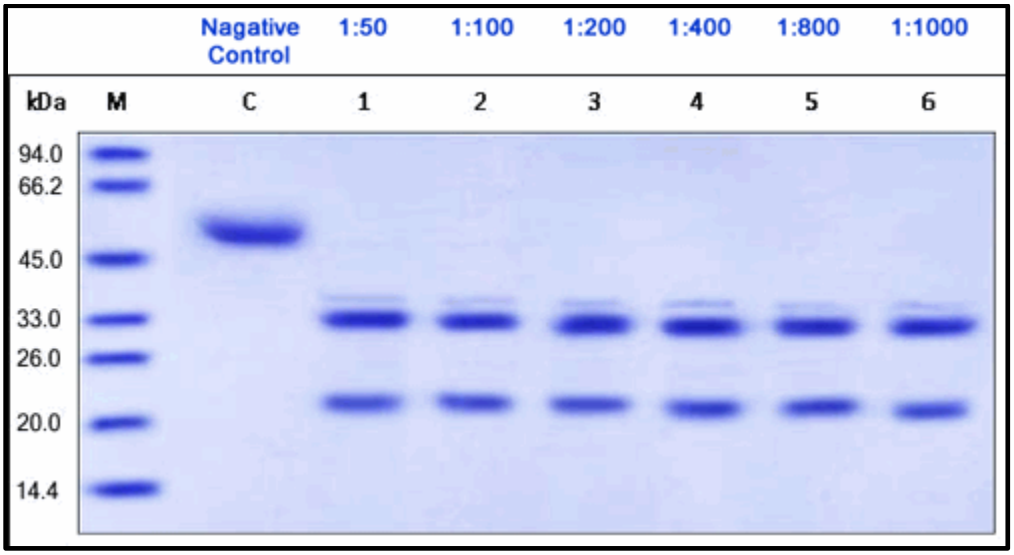
Resuspend the enzyme powder with 50% glyceroal to 2U/µl. Keep reconstituted enzyme at -20oC in aliquots.

Protocol

1. Dilute fusion protein to 1-2 mg/ml with cold Cleavage Buffer. Keep a small aliquot as uncut Negative Control to rule out possible nonspecific cleavage either by autolysis or by contaminated enzymes.
2. Add GST-HRV 3C protease to the target solution at a ratio of 1:100 (u/w) (1 units GST-HRV 3C Protease to 100 µg target protein) as initial cleavage condition. The optimal ratio should be determined empirically. 1:50 to 1:400 works for most target proteins. There is no need to change buffer or dilute HRV-3C Protease.

3. Incubate the reaction mixture at 4°C for 16 hours or overnight. If shorter incubation time is required, more HRV-3C protease should be used or incubate at higher temperature, e.g. RT. It is recommended to test at a small scale, then scale up using the best condition.
4. Remove GST-HRV 3C Protease by passing cleaved protein through a GE Glutathione Sepharose 4B column.
5. Analyze cleavage efficiency with SDS-PAGE.

A sample protein cleavage result was shown below:



A 52kD protein was treated with HRV-3C at different protein/enzyme ratios for 16 h at 4 °C.