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

Contents

YCP1208	1.0 KU	Recombinant HRV 3C Protease Lyophilized powder
YCZ1001	100 µg	Control Protein containing 3C cleavage site.
YCZ1002	25 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

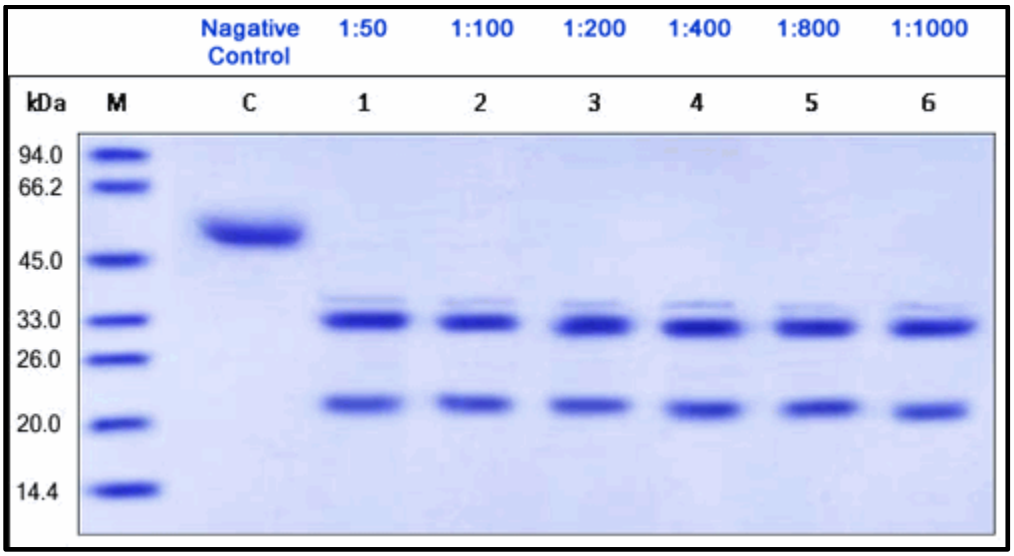
Resuspend the enzyme powder with 50% glyceroal to 2U/µl. Keep reconstituted enzyme at -20oC in aliquots.

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.