HRV 3C Protease

T9800

Storage

Store at 4-8°C for a 1 year in lyophilized state.
Stable at 4-8°C for 1 month under sterile conditions after reconstitution.
-20°C to -70°C for 3 months under sterile conditions after reconstitution.
Avoid repeated freeze-thaw cycles.

Contents

- Product Manual
- HRV 3C Protease
- HRV 3C Cleavage Control Protein
- 10X HRV 3C Cleavage Buffer

Shipping Condition

Ship on ice pack.

Description

HRV 3C Protease is fusion protein of GST and human rhinovirus (HRV) type 14 3C protease. Substrate recognition and cleavage are likely to be dependent not only upon primary structural signals, but also upon the secondary and tertiary structures of the fusion protein as 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 stronlgy stimulated by high concentration of anions such as sulfate.

Source

Recombinant HRV-3C Protease is a recombinant form of human rhinovirus (HRV) type 14 3C protease (11kDa on SDS-PAGE) produced in Escherichia coli cells.

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.

Specificity

The enzyme recognizes the cleavage site: Leu-Glu-ValLeu-Phe-Gle-↓-Gly-Pro

1X Cleavage buffer

50mM Tris-HCl, pH 7.0 (at 25°C), 150mM NaCl, 1mM EDTA, 1mM dithiothreitol.
Chill to 5°C prior to use.

Formulation

Lyophilized from 0.22um filtered solution in 50mM Tris, 150mM NaCl, 1mM EDTA and 0.05% Tween 20.

Reconstitution

It is recommended to reconstitute the lyophilized HRV 3C Protease in sterile 50% glycerol (1,000ul), then the final concentration is 1U/ul. It is recommended to reconstitute the HRV3C Cleavage Control Protein in 100ul 1X HRV 3C Cleavage Buffer.

Unit Definition

> 1 Unit/ug. One unit will cleave >95% of 100ug fusion control protein in 50mM Tris-HCl, 150mM NaCl, pH 7.5 at 4°C for 16 h.

Protocol

Cleavage Protocol:

1. Make fresh cold cleavage buffer, recommended typical cleavage buffer is 50mM Tris-HCl, pH 7.0 (at 25°C), 150mM NaCl, 1mM EDTA, 1mM dithiothreitol. Cleavage buffer should be a buffer in which the target protein is soluble. There should be a buffer. The cleavage buffer should be compatible with downstream purification processes. e.g. minimal amount of EDTA or DTT if Ni column will be used to remove the cleaved His-tag. HRV-3C protease is compatible with 500mM NaCl and 400mM imidazole.
2. Dilute the fusion protein pool to 1-2mg/ml with cold Cleavage Buffer. This is optional in case the target protein aggregates in the buffer. Keep a small aliquot as Uncut sample (Native control) to detect a possible unspecific cleavage either by autolysis or by proteolytic contaminations of the fusion protein.
3. Add HRV3 protease at a Protease: Target protein ratio of 1:100 (w/w) (1,000 unit HRV 3C Protease to 100mg target protein) as initial cleavage condition. The optimal ratio should be determined empirically. A Protease-to-target protein ratio (w/w) of 1:50 to 1:400 should work for most target proteins. There is no need to change buffer or dilute HRV-3C Protease.
4. Incubate the reaction mixture at 4°C for 16 hours or overnight. If shorter incu -bation time is required, more amount of HRV-3C protease or higher temperature (RT) should be implemented. When the cleavage conditions are optimized at a small scale, scale up the cleavage proportionally according to specific application requirement.

Removal of HRV GST-3C Protease:

1. Use Glutathione Agarose High Flow to remove the HRV 3C Protease.
2. If desired, determine and compare the extent of cleavage of the samples by SDS-PAGE analysis.

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