MCLAB
Molecular Cloning Laboratories

# User Manual

Version 3.2

## Product name: HRV3C Protease

## Cat #: HRV3-100, HRV3-200, HRV3-OEM, B-CB10

## Description:

MCLAB's HRC 3C Protease is fused to GST, so that the fusion tag can be easily removed from the protein by cleavage reactions using immobilized glutathione (GSH). HRV 3C Protease is highly active at 4°C, and also active in a variety of commonly used buffers, providing more flexibility in experimental design to keep the activity and intact structure of the target proteins.

## Applications:

HRV 3C Protease is a recombinant cysteine protease used to remove fusion tags from proteins with the HRV 3C cleavage sequence. HRV 3C Protease is highly specific for the PreScission Site, Leu-Glu-Val-Leu-Phe-Gln-↓-Gly-Pro, and cleaves between the Gln and Gly residues.

**Supplied With:**

HRV 3C Substrate, 100 µl (1 mg/mL)

10x HRV3C Protease Reaction buffer (10 mL)

**HRV3C Protease 10X Cleaveage Buffer:**

500 mM Tris-HCl (pH 7.0)

1.5 M NaCl

10 mM EDTA

10 mM DTT

## Protocol:

**Cleavage Reaction In-Solution**

1. Prepare the cleavage reaction by adding the previously purified target protein into 10X HRV 3C Reaction Buffer

diluted to 1X.
Note: HRV 3C protease is active in a variety of different buffers.
Optimize the enzyme: substrate ratio when using other purification buffers.

2. Add 1µL (2 units) of HRV 3C protease to the reaction for each up to 200 µg of the fusion protein.

Note: Test the proper enzyme:substrate ratio on a small scale before scale-up. Use a protease-to-target protein ratio from 1:50 to 1:200.

3. Incubate the cleavage reaction overnight at 4°C for complete cleavage.

Note: Completion of the cleavage reaction maybe monitored at different time points by removing a small portion of the reaction to run on an SDS-PAGE gel.