Lot # XXXXX

BostonBiochem

MATERIAL DATA SHEET

Papain-Like Protease, *SARS virus recombinant*
Cat. # E-610

The Papain-like protease ("PLPro") from the human SARS coronavirus (Severe Acute Respiratory Syndrome coronavirus) is a cysteine protease located within the non-structural protein 3 (NS3) section of the viral polypeptide. PLPro activity is required to process the viral polyprotein into functional, mature subunits; specifically, PLPro cleaves a site at the amino-terminus of the viral replicase region. In addition to its role in viral protein maturation, PLPro possesses a deubiquitinating and deISGylating activity. *In vivo*, this protease antagonizes innate immunity by inhibiting IRF3-induced production of type I interferons. PLPro has been reported to hydrolyze both K48- and K63 linked poly-Ubiquitin chains *in vitro*. When used at low concentrations, the enzyme demonstrates a strong preference for K48-linked tetra-Ubiquitin chains which are primarily converted to di-Ubiquitin species. This protein contains an N-terminal 6-His tag..

Product Information	
Quantity:	50 µg
Stock:	X mg/ml (X µM) in 50 mM HEPES pH 7.5, 100 mM NaCl, 2 mM TCEP.
MW:	37 kDa
Purity:	> 95% by SDS-PAGE under reducing conditions and visualized by Colloidal Coomassie Blue stain

Use & Storage	
Use:	Recombinant SARS virus PLPro is a Ubiquitin- and ISG15-deconjugating enzyme. Reaction conditions will need to be optimized for each specific application. We recommend an initial PLPro concentration of 20-100 nM when using Ubiquitin-AMC or Ubiquitin-Rh110 (**U-550**, **U-555**) substrates. Using tetra-Ubiquitin chains as a substrate, PLPro demonstrates a preference for K48 linkages when using a 100 nM enzyme concentration—at higher enzyme concentration other linkages are cleaved as well.
Storage:	Store at -80°C. Avoid multiple freeze/thaw cycles.

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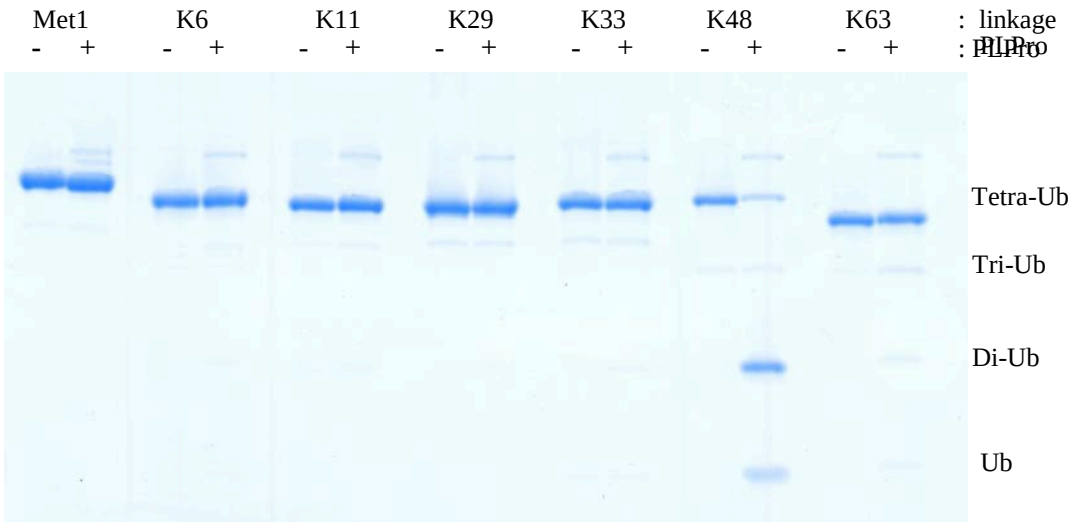
Example Data



SARS PLPro preferentially cleaves K48-linked tetra-Ubiquitin

2 µg (3µM) of tetra-Ubiquitin was incubated with 100 nM PLPro in 20 µl reactions buffered with 50 mM HEPES pH 8, 100 mM NaCl and 2 mM TCEP. After 45 minutes at 37°C, reactions were quenched with reducing Sample Buffer and analyzed using 18% SDS-PAGE gels and Colloidal Coomassie Blue stain.

Literature

References:

Frieman M., *et al.* (2009) J. Virol. **83**: 6689
Lindner H.A., *et al.* (2007) Arch. Biochem. Biophys. **466**: 8
Ratia K., *et al.* (2014) PLoS Pathog doi:10.1371/journal.ppat.1004113
Russell N., *et al.* (2016) FASEB Ubiquitin & Cell Reg. (poster)

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Rev: 2/27/2017

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