SARS-CoV-2-related Protease Assays

TARGET SPECIFIC ASSAY SERVICES

Reaction Biology developed assays to investigate the inhibition of proteases that are essential for the replication cycle of SARS-CoV-2, the novel coronavirus responsible for COVID-19. These assays are being offered to enable timely discovery and development of COVID-19 therapeutics:

- Furin
- Cathepsin L
- PLpro
- Mpro
- TMPRSS2
- S protein – ACE2 binding assay (here)

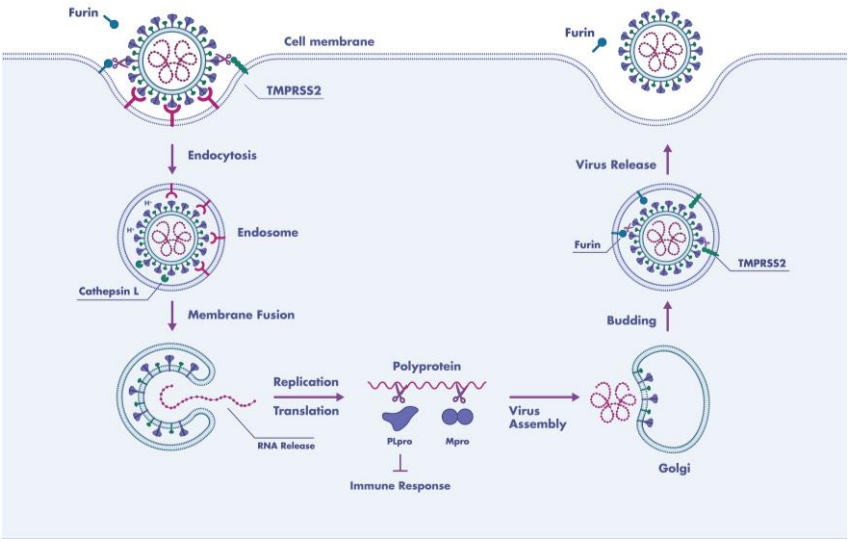

Figure 1: Proteases play a critical role in the life cycle of SARS-CoV-2. *Host proteases Furin and TMPRSS2 play a role in the pathogenesis and replication of respiratory viruses and cleave the spike protein to enable membrane fusion for virus entry. Cathepsin L further primes the spike protein in the acidic environment of endosomes. PLpro and Mpro are viral proteases required for virus replication. They proteolytically process the polyprotein, which is a prerequisite for virus assembly. PLpro is also known to interfere with the anti-viral immune response of the host cell.*

➢ PLpro (Accession #YP_009725299.1)

The papain-like protease (PLpro) is responsible for cleavage of the coronavirus polyprotein necessary for virus assembly. PLpro also suppresses the host innate immune through the reversal of ubiquitination and ISGylation events. Inhibition of PLpro is a potential drug target to prevent SARS-CoV-2 replication and interfere with the immune-suppressing activites of the virus.

- Viral protein: Accession # YP_009725299.1

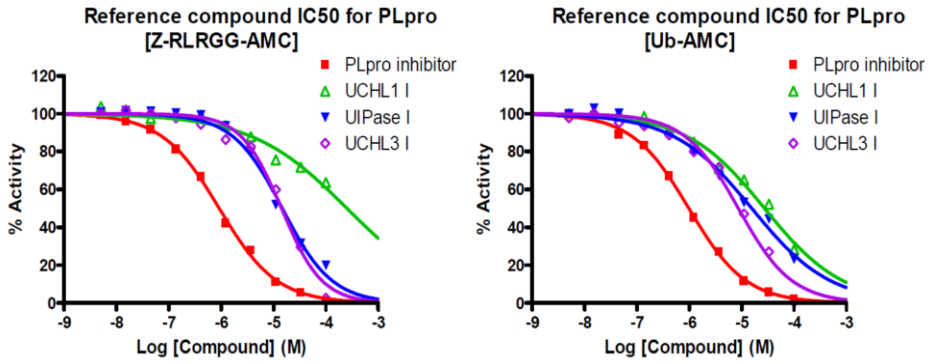

Figure 2.: Example data. *The PLpro assay allows for screening compounds using a fluorogenic substrate, either Z-RLRGG-AMC or Ubiquitin-AMC. PLpro cleaves the substrate for the release of AMC and the resulting fluorescence can be quantified with a luminometer for readout of PLpro enzymatic activity.*

➢ Cathepsin L (Uniprot entry: P07711)

Cathepsin L is an endosomal cystein protease. It mediates cleavage of the coronavirus surface spike glycoprotein S1 subunit. Cleavage of the S1 subunit is required for coronavirus entry into host cells, endosome membrane fusion of the virus and host cell, and viral RNA release. Inhibition of cathepsin L is a potential drug target to block the entry of SARS-CoV-2 into host cells.

The cathepsin L assay allows for screening potential inhibitory compounds using a fluorogenic peptide, Z-FR-AMC. Hydrolysis of the Arg-AMC amide bond releases AMC, a highly fluorescent group which can be detected with the EnVision fluorescence reader.

➢ Mpro (Accession #QHD43415.1)

The viral protease Mpro, also called 3CLpro) plays an essential role in the coronavirus replication by processing the polyproteins that are translated from the viral RNA. It operates 11 cleavage sites on the large polyprotein 1ab with a recognition sequence that has no similarity with human proteases which makes Mpro a promising target for new COVID-19 therapeutics since toxic side effects will be unlikely.

The Mpro assay allows for screening of potential inhibitory compounds using a peptide substrate that can be cleaved by Mpro. The FRET peptide contains a quencher and a donor thus fluorescence is quenched in an intact peptide. Upon hydrolyzation of the peptide the donor fragment generates fluorescence for quantification of Mpro activity.

➢ Furin (Uniprot entry: P09958)

Furin is a serine endoprotease important for the proteolytic processing of proteins within the secretory pathway, including pathogen-derived proteins. Furin is the proprotein convertase (PPC) that preactivates the SARS-CoV-2 spike and enhances entry into cells and thus a potential drug target for the inhibition of furin-dependent virus replication.

The furin assay can screen potential inhibitory compounds using a fluorogenic peptide, pERTKR-AMC. Hydrolysis of the Arg-AMC amide bond releases 7-amino-4-methylcoumarin (AMC), a highly fluorescent group detectable with the EnVision fluorescence reader, see figure 2.

➢ TMPRSS2 (Uniprot entry: O15393)

Besides its high expression in the prostate, TMPRSS2 is also expressed in the respiratory and gastrointestinal tract facilitating cleavage of the Coronavirus SARS-CoV-2 S protein to prime it for membrane fusion to promote virus entry. The protease was also found important in the activation of glycoproteins of other Coronaviruses and other respiratory viruses such as influenza, parainfluenza, and Sendai virus.

Our TMPRSS2 assay is available for screening of potential inhibitory compounds using the fluorogenic peptide Boc-Gln-Ala-Arg-AMC. Hydrolysis of the Arg-AMC amide bond releases 7-amino-4-methyl coumarin (AMC), a highly fluorescent group detectable with the EnVision fluorescence reader, see figure 2.