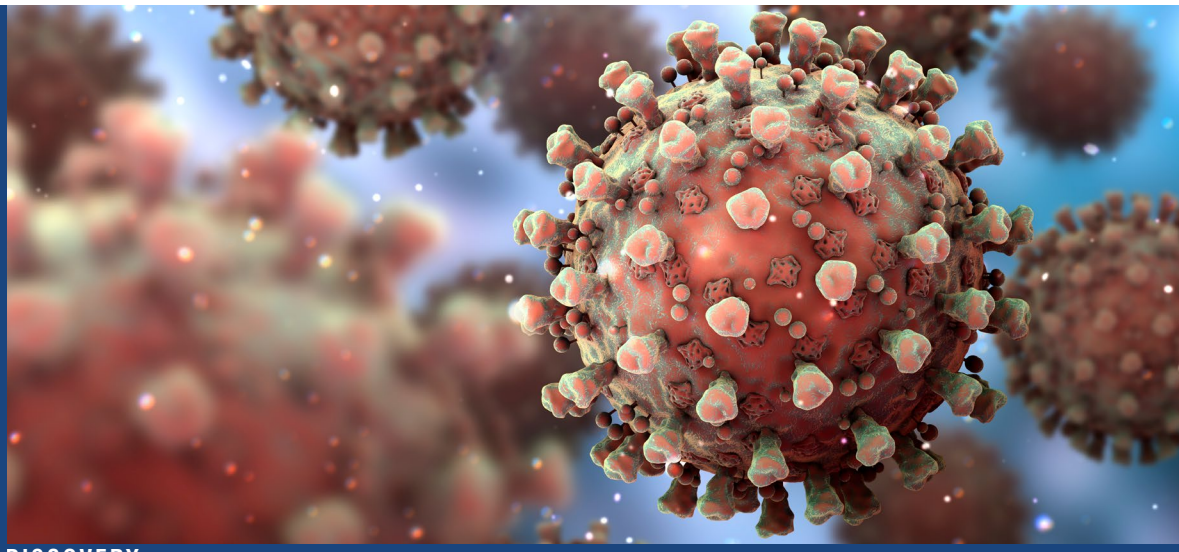
charles river

Summary

Viral proteases have been validated for several viral targets including HIV and HCV. This protease inhibition assay, specific to COVID-19, can be used to screen compounds as potential target for antiviral therapies.



DISCOVERY

Complex Biology *In Vitro* Assay

Viral COVID-19 Protease Inhibition Assay

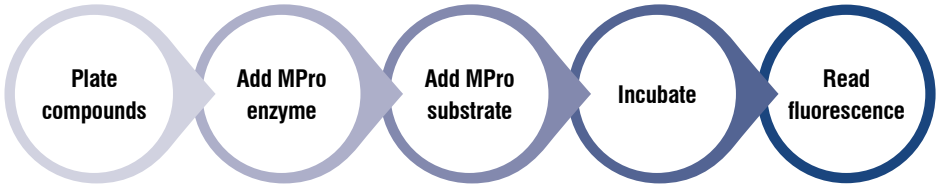
Proteases have been found in almost every organism and play a part in many cellular processes by catalyzing the proteolytic cleavage of specific target sequences. Viruses use proteases to process strings of viral precursor proteins into mature components of the viral machinery, such as structural proteins of the viral capsid, replicases and other non-structural proteins. The central function of viral proteases, makes them ideal targets in small molecule inhibitors screening. Indeed, protease inhibition has been proven effective in antiviral therapies applied to, diseases such as HIV.

A validated and robust *in vitro* assay has been developed in a 384-well format using a COVID-19 main protease expressed in *E. Coli* cells. Proteolytic cleavage of a peptide substrate conjugated with a fluorophore (Edans) and a quencher (DABCYL) causes robust and measurable fluorescence emission directly proportional to the protease activity. Compounds identified as hits through this screening process could then be evaluated in downstream models for potential antiviral therapies for COVID-19.

Assay Principle

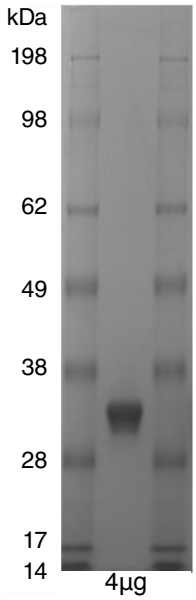
COVID-19 main protease (Uniprot: P0DTD1 (R1AB_SARS2) residues (3264-3569)) is expressed in *E. coli* followed by in house purification on nickel-sepharose resin. The purified protein is used in a 384-well assay to cleave and activate a fluorescently tagged substrate. Time-dependent fluorescence emission is therefore used as indication of protease activity. Protease inhibitors will prevent substrate cleavage, indicative of inhibition of COVID-19 protease decreasing the rate of fluorescence emission over time.

Assays are performed in 384-well format, and compounds tested as ten-point concentration response curves. A larger number of compounds can be screened in single point format, followed by potency determination of hits.

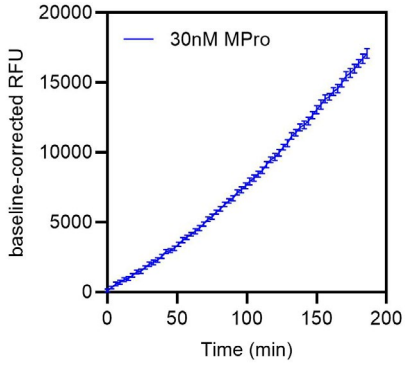


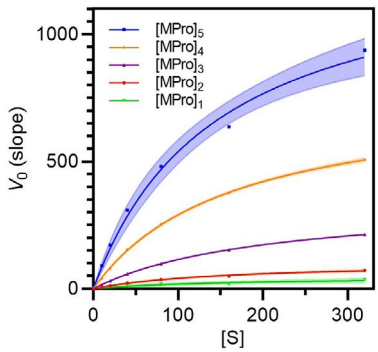
Assay Setup

Test compounds	8-10-point concentration response curves (in biological duplicate) normalized to 1% DMSO
Enzyme	30-50nM COVID-19 MPro Uniprot: P0DTD1 (R1AB_SARS2) residues (3264-3569)
Substrate	200-300μM; based on target motif [ILMVF]-Q-|-[SGACN]; modified with DABCYL/ Glu(EDANS) quenched FRET pair.
Incubation time	from 30 minutes to 3 hours
Readout	Edans fluorescence



Sars-CoV-2 Protease-His$_6$ (MPro)
Uniprot: P0DTD1 (R1AB_SARS2)
residues (3264-3569)





Summary

The MPro assay allows quick and robust screening of small molecules in a convenient 384-well format for high-throughput screening. It uses purified COVID-19 protease, with activity verified in-house, ensuring consistency and flexibility for every research project. The assay can be used to test selected client molecules or to screen the available Charles River libraries. Clients can then refine initial hits using Charles River's in-house *in vitro* DMPK assays and medicinal chemistry services.

Assay Reference Code

OTS301 COVID-19 Protease Inhibition Assay

charles river
askcharlesriver@crl.com • www.criver.com