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Datasheet for 200-401-A51

SARS 3Cl Protease Antibody

Overview

Description:	Anti-SARS-CoV 3CL Protease (RABBIT) Antibody - 200-401-A51
Item No.:	200-401-A51
Size:	500 µg
Applications:	WB
Reactivity:	Virus
Host Species:	Rabbit

Product Details

Background:	Generally, viruses have proteases to process their proteins into active form. Because of its pivotal role in the viral life cycle, proteases are primary targets for the development of antiviral agents. 3CL protease, a viral cysteine proteinase, plays an important role in co-translational proteolytic processing of Coronavirus polyproteins. The 3CL protease cleaves as much as 11 sites in the replicase polyproteins and also releases the key replicative functions of polymerase and helicase. 3CL protease is the only Coronavirus protein for which structural information is available. 3CL protease comprises three domains, the substrate-binding site is expected to be located between domains I and II, and domain III is a globular cluster comprising five helices. 3CL protease is a homodimer. Anti-SARS-CoV 3CL Protease Antibody is useful for researchers interested in viral research.
Synonyms:	rabbit anti-Sars CoV 3CL Protease Antibody, 3CL PRO antibody, 3CLp antibody, nsp5 antibody
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	3CL-PRO
Reactivity:	Virus
Immunogen Type:	Recombinant Protein

Immunogen:	This protein A purified antibody was prepared from whole rabbit serum produced by repeated immunizations with a purified recombinant protein corresponding to full length SARS Coronavirus 3CL Protease. Lifesensors Inc. (www.lifesensors.com) prepared the 3CL Protease as follows: SUMO-3CL protease fusion was expressed in E. coli in LB medium and purified by Ni-NTA resin affinity chromatography (Qiagen). After the fusion was cleaved by the SUMO Protease (LifeSensors), the SUMO tag and protease were subtracted from the 3CL protease using MAC and the 3CL protease was finally purified using Anion Exchange Chromatography with the Macro-Prep High Q resin (BioRad) and size exclusion chromatography.
Purity/Specificity:	This protein A purified antibody is directed against SARS Coronavirus 3CL Protease. The product was purified from monospecific antiserum by protein A affinity purification. BLAST analysis was used to suggest reactivity with related Coronavirus proteins. Cross reactivity with homologues from other sources has not been determined.
Relevant Links:	• NCBI - 29837498 • UniProtKB - P0C6U8 • GeneID - 1489680

Application Details

Tested Applications:	WB
Application Note:	This protein A purified antibody has been tested for use in ELISA and by western blot. Specific conditions for reactivity should be optimized by the end user. Expect a band approximately 34 kDa in size corresponding to SARS 3CL Protease by western blotting in the appropriate cell lysate or extract.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:10,000 - 1:50,000
IF:	User Optimized
WB:	1:2,000 - 1:10,000

Formulation

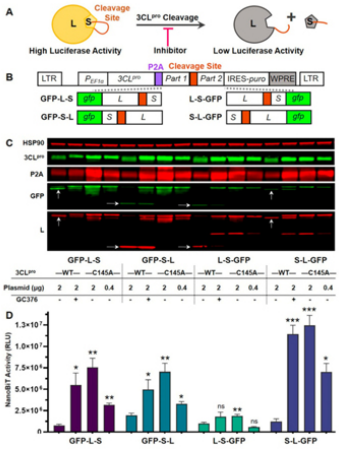
Physical State:	Lyophilized
Concentration:	5.1 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None

Reconstitution Volume:	100 µL
Reconstitution Buffer:	Restore with deionized water (or equivalent)

Shipping & Handling

Shipping Condition:	Ambient
Storage Condition:	Store vial at 4° C prior to restoration. For extended storage aliquot contents and freeze at -20° C or below. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Expiration:	Expiration date is one (1) year from date of receipt.

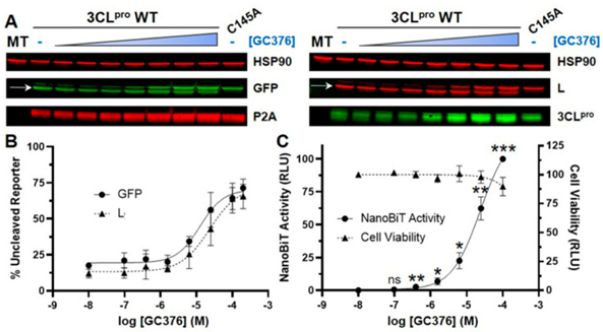
Images



Western Blot

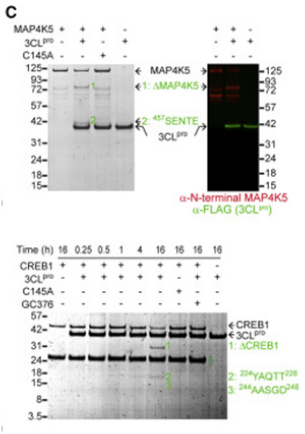
Development of cell-based luciferase complementation reporters to detect inhibition of SARS-CoV-2 3CLpro activity. (A) Strategy for detection of 3CLpro inhibition using NanoBiT, a luciferase complementation reporter comprised of Large BiT (L) and Small BiT (S). When connected by a linker containing a 3CLpro cleavage site, 3CLpro cleavage should result in a loss of complementation and low luciferase activity. Conversely, 3CLpro inhibitors should prevent cleavage, resulting in high luciferase activity. (B) Lentiviral vectors that express 3CLpro and reporters. P2A, a self-cleaving peptide, was inserted between 3CLpro and reporters. Reporters consisted of GFP linked to L and S in various orders. L and S were separated by the SARS-CoV-2 nsp4-nsp5 cleavage site. LTR, long terminal repeat; PEF1α, EF1α promoter; IRES, internal ribosome entry site; puro, puromycin resistance gene; WPRE, woodchuck hepatitis virus post-transcriptional regulatory element. (C) Western blotting to examine reporter expression and cleavage. Lentiviral vectors expressing 3CLpro WT or C145A (a catalytically inactive mutant) were transfected into 293T cells, and DMSO (control) or the 3CLpro inhibitor GC376 (100 µM) was added. Cell lysates were collected 30 h post-transfection. 3CLpro was detected using anti-SARS-CoV 3CLpro or anti-P2A antibodies, whereas reporters were detected using anti-GFP or anti-L antibodies. Arrows denote cleavage products of expected sizes. For 3CLpro C145A, two different plasmid amounts were transfected: 2 or 0.4 µg. HSP90 was included as a loading control. (D) NanoBiT luciferase activity. 293T cells were transfected (same sample order as in C), and NanoBiT activity (expressed as relative luciferase units, or RLU) was measured 30 h post-transfection. The data represent the mean ± standard deviation of four independent experiments; ns: not significant; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (relative to 3CLpro WT without drug; mixed effects analysis with Dunnett's post-test). Fig 1.

PMID: 33498923



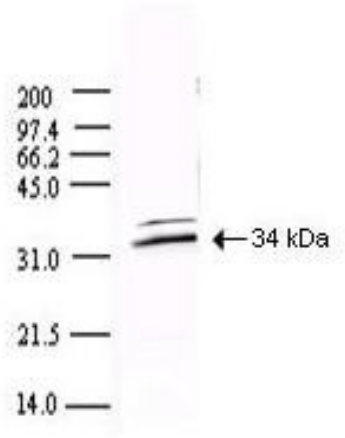
Western Blot

The luciferase complementation assay accurately reflects concentration-dependent inhibition of SARS-CoV-2 3CLpro-mediated cleavage. (A) Western blotting to examine reporter cleavage. 293T cells were transfected with the lentiviral vector containing 3CLpro WT and the S-L-GFP reporter cassette, and DMSO (control) or GC376 (0.1, 0.4, 1.6, 6.3, 25, 100, or 200 µM) was added. Mock transfection (MT) and 3CLpro C145A S-L-GFP were included as controls. On the GFP and LgBiT (L) panels, the bottom band corresponds to cleaved reporter (L-GFP, 46 kDa, indicated by arrows), while the top band corresponds to uncleaved reporter (S-L-GFP, 49 kDa). (B) Quantification of the percentage of uncleaved reporter from three independent western blots based on GFP (circle) or L (triangle) detection. (C) NanoBiT luciferase activity and cell viability. 293T cells were transfected with the S-L-GFP lentiviral vector, and DMSO (control) or GC376 (0.1, 0.4, 1.6, 6.3, 25, or 100 µM) was added. NanoBiT activity (circle, left axis) and cell viability (measured with the CellTiter-Glo 2.0 assay; triangle, right axis) were analyzed 30 h post-transfection and are expressed as relative luciferase units (RLU). Cell viability data were normalized to the DMSO sample, which was set to 100%. NanoBiT data were normalized to the DMSO and 100 µM GC376 samples, which were set to 0 and 100%, respectively. The data represent the mean ± standard deviation of four independent experiments; ns: not significant; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (relative to no-drug; one-way ANOVA with Dunnett's post-test). Fig 2.
PMID: 33498923



Western Blot

(C) SDS-PAGE, Edman sequencing (green) and immunoblot validation of human MAP4K5 and CREB1 substrates incubated with 3CLpro+/− inhibitor GC376, or 3CLpro-C145A (1:5 mol/mol, E:S). ΔMAP4K5 or ΔCREB1, no sequence obtained. Fig 4.
PMID: 34672947



Western Blot
Western blot using Rockland's Protein A Purified anti-SARS CoV 3CL Protease antibody shows detection of a 34-kDa band corresponding to the protein. Approx. 100 ng of protein was loaded for SDS-PAGE and transferred onto nitrocellulose. The blot was incubated with a 1:5,000 dilution of the antibody at room temperature for 1 h followed by detection using IRDye™800 labeled Goat-a-Rabbit IgG [H&L] (611-132-122) diluted 1:10,000. The fluorescence image was captured using the Odyssey® Infrared Imaging System developed by LI-COR. IRDye is a trademark of LI-COR, Inc. Other detection systems will yield similar results.

References

- Pablos I. et al. Mechanistic insights into COVID-19 by global analysis of the SARS-CoV-2 3CLpro substrate degradome. *Cell Rep.* (2021)
- Rawson JMO. et al. Development of a Cell-Based Luciferase Complementation Assay for Identification of SARS-CoV-2 3CLpro Inhibitors. *Viruses* (2021)

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