

### Certificate of Analysis

**Product:** Protein A Purified Anti-SARS-CoV 3CL Protease (Rabbit)

**Code:** 200-401-A51

**Lot #:** 16569

**Size:** 500 µg

**Physical State:** Lyophilized

**Antibody Concentration:** 5.0 mg/ml (by UV absorbance at 280 nm)

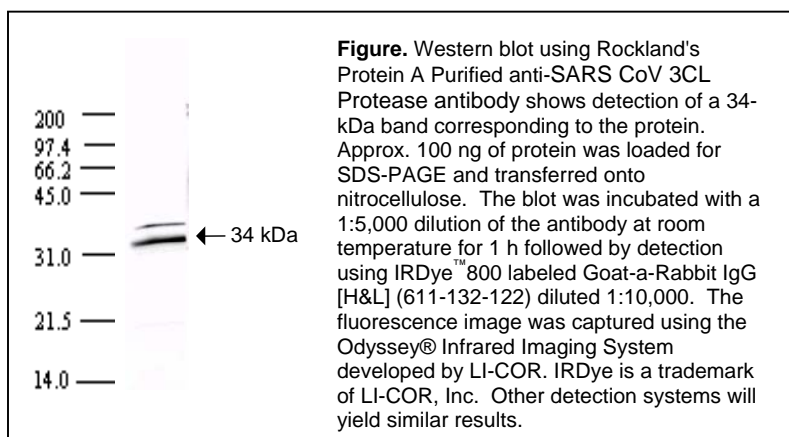
**Buffer:** 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2

**Stabilizer:** None

**Preservative:** 0.01% (w/v) Sodium Azide

**Storage Conditions:** Store vial at 4° C prior to restoration. Reconstitute in 0.1 ml of deionized water or equivalent. Dilute only prior to immediate use. For extended storage aliquot contents and freeze at -20° C or below. Avoid cycles of freezing and thawing. Expiration date is one (1) year from date of opening.

**Background Information:** 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.



**Application Note(s):** 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.

**Recommended Dilutions:**

<b>ELISA</b>	1:10,000 - 1:50,000
<b>WESTERN BLOT</b>	1:2,000 - 1:10,000
<b>IF MICROSCOPY</b>	User Optimized
<b>OTHER APPLICATIONS</b>	User Optimized

**Purity and 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 Link(s):** NCBI Link (polyprotein) [P59641](#) NCBI Link (3CL-PRO) [NP\\_828863](#)

**Protein Sequence:** SARS Coronavirus 3CL Protease, 306 aa, predicted MW 33.8 kDa

1	sgfrkmafps	gkvegcmvqv	tcgtttlngl	wlddtvycpr	hvictaedml	npnyedllir
61	ksnhsflvqa	gnvqlrvigh	smqncllrlk	vdtsnpktpk	ykfvriqpgq	tfsvlacyng
121	spsgvyqcam	rpnhtikgsf	lngscgsvgf	nidydcvsfc	ymhhmelpgt	vhagtdlegk
181	fygpfvdrqt	aqaaagdtti	tlvnlawlya	avingdrwfl	nrfttlnndf	nlvamkynye
241	pltqdhvdil	gplsagtgia	vlmcaalke	llqngmngrt	ilgstiled	ftpdfvvrqc
301	sgvtfq					

**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](http://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.

**General References:**

- Zuo, X., et al. (2005). "Expression and purification of SARS Coronavirus Proteins using SUMO Fusions." *Protein Express Purif* **42**: 100-110.
- Zuo, X., et al. (2005). "Enhanced expression and purification of membrane proteins by SUMO fusion in Escherichia coli." *J Struct Funct Genomics* (In Press).
- Malakhov, M. P., et al. (2004). "SUMO fusion and SUMO-specific protease for efficient expression and purification of proteins." *J Struct Funct Genomics* **5**(1-2): 75-86.
- Butt, T. R. et al. (2005). "SUMO fusion technology for difficult-to-express proteins." *Protein Express Purif* (In Press).
- Marra, M.A., et al. (2003). "The genome sequence of the SARS-associated coronavirus." *Science* **300**(5624): 1399-1404.
- Sun H. et al. (2003). "Molecular cloning, expression, purification, and mass spectrometric characterization of 3C-like protease of SARS coronavirus." *Protein Express Purif* **32**(2): 302-308.
- Xu, X. et al. (2003). "Molecular model of SARS coronavirus polymerase: implications for biochemical functions and drug design." *Nucleic Acids Res.* **31**(24): 7117-7130.
- Ksiazek, T.G. et al. (2003). "A novel coronavirus associated with severe acute respiratory syndrome." *N. Engl. J. Med.* **348**(20): 1953-1966.
- Rota, P.A., et al. (2003). "Characterization of a novel coronavirus associated with severe acute respiratory syndrome." *Science* **300**(5624): 1394-1399.
- Muller, S. et al. (2001). "SUMO, ubiquitin's mysterious cousin." *Nat. Rev. Mol. Cell Biol.* **2**(3) 202-210.
- Melchior, F. (2000). "SUMO--nonclassical ubiquitin." *Annu. Rev. Cell Dev. Biol.* **16**: 591-626.

**Related Products:**

#200-401-A50	Protein A Purified Anti-SARS-CoV (N) Protein (Rabbit)	#200-401-A54	Protein A Purified Anti-SARS-CoV nsp13 (Rabbit)
#200-401-A51	Protein A Purified Anti-SARS-CoV 3CL Protease (Rabbit)	#200-401-A55	Protein A Purified Anti-SARS-CoV (M) Protein (Rabbit)
#200-401-A52	Protein A Purified Anti-SARS-CoV nsp3 (Rabbit)	#611-103-122	HRP Anti-RABBIT IgG (H&L) (GOAT) MX10
#200-401-A53	Protein A Purified Anti-SARS-CoV nsp8 (Rabbit)	#611-132-122	IRDye800 Anti-RABBIT IgG (H&L) (GOAT) MX10
#MB-070	Blocking Buffer for Fluorescent Western Blotting	#KIA-003	<b>MaxTag™</b> Anti-RABBIT IgG Kit for Immunoblotting

**USDA Certification:** All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation.

**Note:** This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information.