

### Certificate of Analysis

**Product:** Anti-SARS-CoV Nonstructural Protein 3 (nsp3) (Rabbit)

**Code:** 100-401-A52

**Lot #:** 17038

**Size:** 100 µl

**Physical State:** Liquid (sterile filtered)

**Protein Concentration:** 85 mg/ml (by Refractometry)

**Buffer:** None

**Stabilizer:** None

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

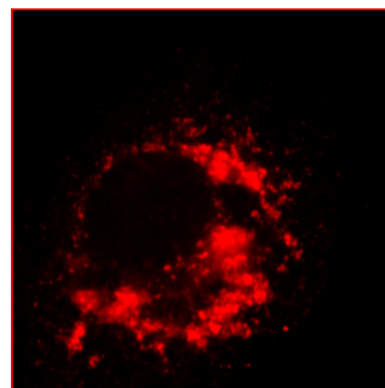
**Storage Conditions:** Store vial at -20° C prior to opening. Dilute only prior to immediate use. For extended storage aliquot contents and freeze at -20° C or below. Avoid cycles of freezing and thawing. Expiration date is one (1) year from date of opening.

**Background Information:** The nonstructural protein 3 (nsp3) is one of the SARS-Coronavirus replicase cleaving products, encoded by ORF1a. Nsp3 is thought to be part of the viral replication complex, which is associated with intracellular membranes. Nsp3 contains a papain-like cysteine proteinase and ADP-ribose-1'-monophosphatase activities.

#### Recommended Dilutions:

<b>IMMUNO PRECIPITATION</b>	1:60
<b>WESTERN BLOT</b>	1:1,000
<b>IMMUNO ELECTRON MICROSCOPY</b>	1:500
<b>IF MICROSCOPY</b>	1:1,000
<b>OTHER APPLICATIONS</b>	User Optimized

**Figure.** Immunofluorescence Microscopy using Rockland Immunochemicals anti-SARS-CoV nsp3 protein antibody, 6-h post infection Vero-E6 cells. For detection Cy3 conjugated Goat-anti-Rabbit IgG MX (611-104-122) was used. *Personal Communication, Eric Snijder, Leiden University Medical Center, Leiden, Netherlands.*



**Application Note(s):** This antibody has been tested for use in immunofluorescence microscopy, immunoelectron microscopy, immunoprecipitation and by western blot. Specific conditions for reactivity should be optimized by the end user. Expect a band of approximately 211 kDa in size corresponding to SARS-CoV nsp3 by western blotting in the appropriate cell lysate or extract. For immunofluorescence microscopy, Vero-E6 cells, grown on glass slides, were infected with SARS-CoV-Fr1 strain for 1 h at 37°C. Infection occurred in PBS/DEAE/2%FCS followed by exchange to EMEM/25mMHEPES/2%FCS. Cells were fixed with PBS/3%PFA. After washing fixed cells, antibody incubation was performed in PBS/5%FCS for 30 min.

**Purity and Specificity:** This antibody is directed against SARS-Coronavirus nsp3 protein. The product is neat antiserum. Cross reactivity with homologues from other sources has not been determined.

**Relevant Link(s):** NCBI Link (polyprotein) [NP\\_828849](#) NCBI Link (nsp3) [NP\\_828862](#)

**Immunogen:** This whole rabbit serum was produced by repeated immunizations with 3 *E.coli* derived proteins representing different parts of the SARS-CoV Nsp3 protein: aa 834-943 of polyprotein 1a (ADRP domain), aa 1208-1557 of polyprotein 1a (SARS-Unique Domain) and aa 1558-2040 of polyprotein 1a (Papain-like proteinase domain).

**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.

**Protein Sequence:** SARS Coronavirus Nonstructural Protein 3, 1922 aa, predicted MW 211 kDa

1	apikgvtfge	dtwewvqgyk	nrvitfelde	rvdkvlnek	svytvesgte	vtefacvvae
61	avvktlqpv	dlltnmgidl	dewsvatfyl	fddageenfs	srmycsfypp	deeeeddaec
121	eeeeidetce	heygteddyq	gplefgasa	etvrveeeee	edwlddtteq	seiepepept
181	peepvnqftg	ylktdnvai	kcdivkeaq	sanpmvivna	anihlkhggg	vagalnkatn
241	gamqkesddy	iklngplvtg	gscllsgnhl	akkclhvvgp	nlngediql	lkaayenfns
301	qdillaplls	agifgakplq	slqvcvqtr	tqvviavndk	alyeqvmdy	ldnlkprvea
361	pkqeepnte	dskteeksvv	qkpvdvkpi	kacidevttt	leetkfltnk	lllfadingk
421	lyhdsqnmrl	gedmsflekd	apymvgdvt	sgditcvvip	skkagggttem	lsralkkvpv
481	deyittypgq	gcagytleea	ktalkkcksa	fyvlpseapn	akeeilgtvs	wnlremlaha
541	eetrklmpic	mdvraimati	qrkykgikiq	egivdygvrf	ffytskepva	siitklnsln
601	eplvtmpigy	vthgfnleea	arcmrslkap	avsvsspda	vttyngylts	ssktseehfv
661	etvslagsyr	dwsysgqrte	lgveflkrqd	kivyhtlesp	vefhldgevl	sldklkslls
721	lrevtkikvf	ttvdntnlht	qlvdmstyq	qqfgptyldg	advtkikphv	nhegkttfvl
781	psddtlrsea	feyyhldes	flgrymsaln	htkkwkfpqv	ggltsikwad	nncylssvll
841	<b>alqqlevkfn</b>	<b>apalqeayyr</b>	<b>aragdaanf</b>	<b>alilaysnkt</b>	<b>vgelgdvret</b>	<b>mthllqhanl</b>
901	<b>esakrvlnv</b>	<b>lthcgcgqkttt</b>	<b>ltgveavmym</b>	<b>gtlsydnlkt</b>	<b>gvsipccvgr</b>	<b>datqylvqqe</b>
961	ssfvmsapp	aeyklqqgtf	lcaneytgn	qcgthythita	ketlyridga	hltkmseykg
1021	pvtadvfyket	sytttikpvs	yklgdvtyte	iepkldgyyk	kdnayteqp	idlvptqplp
1081	nasdfnklt	csntkfaddl	nqmtgftkpa	srelsvtffp	dlngdvvaidd	yrhysasfkk
1141	gakllhkpiv	whinqattkt	tfkpwntwclr	clwstkpvdtd	snsfevlave	dtqgmdnlac
1201	esqqpts <b>eev</b>	<b>venptiqkev</b>	<b>iecdvkttev</b>	<b>vgnvilkpsd</b>	<b>egvkvqtqelg</b>	<b>hedlmaayve</b>
1261	<b>ntsitiikkpn</b>	<b>elslalgltk</b>	<b>iathgiaain</b>	<b>svpwskilay</b>	<b>vkpflgqaai</b>	<b>ttsncakrla</b>
1321	<b>qrvfnnypmy</b>	<b>vtllflqlct</b>	<b>ftkstnsrir</b>	<b>aslpptiakn</b>	<b>svksvakicl</b>	<b>daginyvksp</b>
1381	<b>kfsklftiam</b>	<b>wllllsiclg</b>	<b>sllicvtaafg</b>	<b>vllsnfgaps</b>	<b>ycngvrelyl</b>	<b>nssnvtmqmf</b>
1441	<b>cegsfpcsic</b>	<b>lsgldsltsy</b>	<b>paletiqvti</b>	<b>ssykldtil</b>	<b>glaewvlay</b>	<b>mlftkfyll</b>
1501	<b>glsaimqvff</b>	<b>gyfashfisn</b>	<b>swlmwfiisi</b>	<b>vqmapvsamv</b>	<b>rmyiffasfy</b>	<b>yiwksyvhim</b>
1561	<b>dgctsstcmm</b>	<b>cykrnratr</b>	<b>ectivngmk</b>	<b>rsfyvyang</b>	<b>rgfckthnwn</b>	<b>clncdtfctg</b>
1621	<b>stfisdevar</b>	<b>dlslqfkrpi</b>	<b>nptdqssyiv</b>	<b>dsvavkngal</b>	<b>hlyfdkagqk</b>	<b>tyerhplshf</b>
1681	<b>vnldnlrann</b>	<b>tkgslpinvi</b>	<b>vfdgkskcde</b>	<b>saksasvyy</b>	<b>sqlmcpill</b>	<b>ldqalvsdvg</b>
1741	<b>dstevsvmkf</b>	<b>dayvdtsat</b>	<b>fsvpmeklka</b>	<b>lvatahsela</b>	<b>kgvaldgvl</b>	<b>tfvsaarqgv</b>
1801	<b>vtdvtdtkdv</b>	<b>iecklshhs</b>	<b>dlevtgdsnc</b>	<b>nfmltynkve</b>	<b>nmtprldgac</b>	<b>idcnarhina</b>
1861	<b>qvakshnvs</b>	<b>iwnvkdymsl</b>	<b>seqlrkqirs</b>	<b>aakknnipfr</b>	<b>lcattrqvv</b>	<b>nvittkislk</b>
1921	<b>gg</b>					

**General References:**

Snijder, E. J., P. J. Bredenbeek, J. C. Dobbe, V. Thiel, J. Ziebuhr, L. L. M. Poon, Y. Guan, M. Rozanov, W. J. M. Spaan, and A. E. Gorbalenya. 2003. Unique and conserved features of genome and proteome of SARS-coronavirus, an early split-off from the coronavirus group 2 lineage. *J. Mol. Bio.* **331**:991-1004.

Prentice, E., J. McAuliffe, X. T. Lu, K. Subbarao, and M. R. Denison. 2004. Identification and characterization of severe acute respiratory syndrome coronavirus replicase proteins. *J. Virol.* **78**:9977-9986.

Harcourt, B. H., D. Jukneliene, A. Kanjanahaluethai, J. Bechill, K. M. Severson, C. M. Smith, P. A. Rota, and S. C. Baker. 2004. Identification of severe acute respiratory syndrome coronavirus replicase products and characterization of papain-like protease activity. *J. Virol.* **78**:13600-13612.

Putics, A., Filipowicz, W., Hall, J., Gorbalenya, A.E., and Ziebuhr, J. 2005. ADP-ribose-1<sup>st</sup>-monophosphatase: a conserved coronavirus enzyme that is dispensable for viral replication in tissue culture. *J. Virol.* **79**:12721-12731.

Snijder, E.J., van der Meer, Y., Zevenhoven-Dobbe, J.C., Onderwater, J.J.M., van der Meulen, J., Koerten, H.K., and Mommaas, A.M. 2006. Ultrastructure and origin of membrane vesicles associated with the SARS-coronavirus replication complex. Manuscript in preparation.

**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

**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.