ROCKLAND
Immunochemicals for Research

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Certificate of Analysis

Product: Anti-SARS-CoV Nonstructural Protein 8 (nsp8) (Rabbit)

Code: 100-401-A53

Lot #: 17040

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

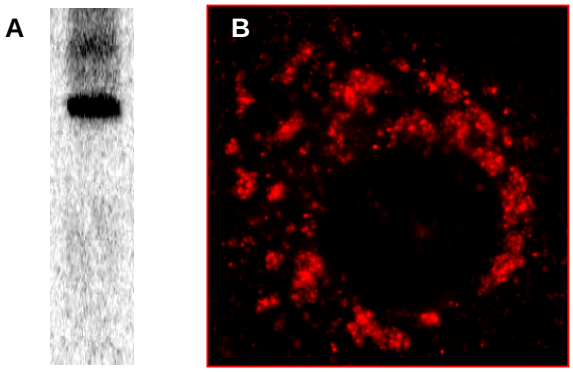
Storage Conditions: Store vial at -20° C prior to opening. Dilute only prior to immediate use. For extended storage aliquot contents and freeze at -20° C or below. Avoid cycles of freezing and thawing. Expiration date is one (1) year from date of opening.

Background Information: The nonstructural protein 8 (nsp8) is one of the SARS-Coronavirus replicase cleaving products, encoded by ORF1a. Nsp8 is thought to be part of the viral replication complex, which is associated with intracellular membranes. No specific information on the function of nsp8 is available.

Recommended Dilutions:

IMMUNO PRECIPITATION	1:60
WESTERN BLOT	1:1,000
IMMUNO ELECTRON MICROSCOPY	1:100
IF MICROSCOPY	1:300
OTHER APPLICATIONS	User Optimized

Figure. Immunoprecipitation followed by western blotting using Rockland Immunochemicals Anti-nsp8 shows a predominant band at 21.8 kDa corresponding to full length SARS protein (panel A). Immunofluorescence Microscopy using anti-nsp8 6-h post infection of Vero-E6 cells (Panel B). 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 22 kDa in size corresponding to SARS-CoV nsp8 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 nsp8 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 (nsp8) NP_828866

Immunogen: This whole rabbit serum was produced by repeated immunizations with a purified His- tagged recombinant protein corresponding to full-length SARS-Coronavirus nsp8.

Protein Sequence: SARS Coronavirus Non Structural Protein 8, 198 aa, predicted MW 21.8 kDa

1	aiasefsslp	syaayataqe	ayeqavangd	sevvlkklkk	slnvaksefd	rdaamqrkle
61	kmadqamtqm	ykqarsedkr	akvtsamqtm	lftmlrkldn	dalnniinna	rdgcvplnii
121	plttaaklmv	vvpdygtykn	tcdgntftya	salweiqqvv	dadskivqls	einmdnspnl
181	awplivtalr	ansavklq				

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.

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	***Max*Tag**™ 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.