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Datasheet for 100-401-A53

Sars-Cov Nonstructural Protein 8 Antibody

Overview

Description:	Anti-SARS-CoV Nonstructural Protein 8 (nsp8) (RABBIT) Antibody - 100-401-A53
Item No.:	100-401-A53
Size:	100 µL
Applications:	IF, WB
Reactivity:	SARS-CoV
Host Species:	Rabbit

Product Details

Background:	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. Anti-SARS-CoV Nonstructural Protein 8 (nsp8) Antibody is useful for researchers interested in viral research.
Synonyms:	rabbit anti-Sars-Cov Nonstructural Protein 8 Antibody, Replicase polyprotein 1a, ORF1a polyprotein, nsp8
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	Antiserum

Target Details

Gene Name:	1a
Reactivity:	SARS-CoV
Immunogen Type:	Recombinant Protein
Immunogen:	This whole rabbit serum was produced by repeated immunizations with a purified His- tagged recombinant protein corresponding to full-length SARS-Coronavirus nsp8.
Purity/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 Links:	• NCBI - 30124074 • UniProtKB - P0C6U8 • GeneID - 1489680

Application Details

Tested Applications:	IF, WB
Application Note:	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/25mM HEPES/2% FCS. Cells were fixed with PBS/3% PFA. After washing fixed cells, antibody incubation was performed in PBS/5% FCS for 30 min.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
IF:	1:300
IP:	1:60
WB:	1:1,000

Formulation

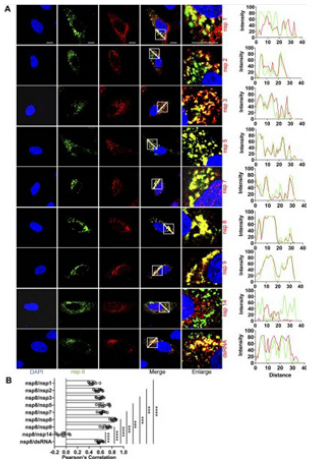
Physical State:	Liquid (sterile filtered)
Concentration:	85 mg/mL by Refractometry
Buffer:	None
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None

Shipping & Handling

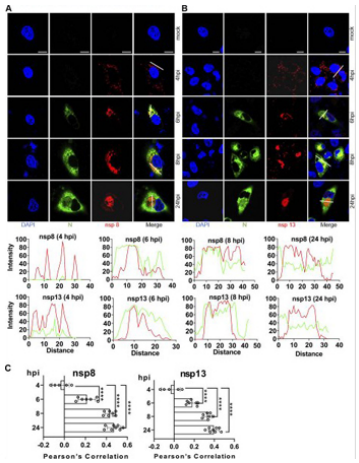
Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several 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.

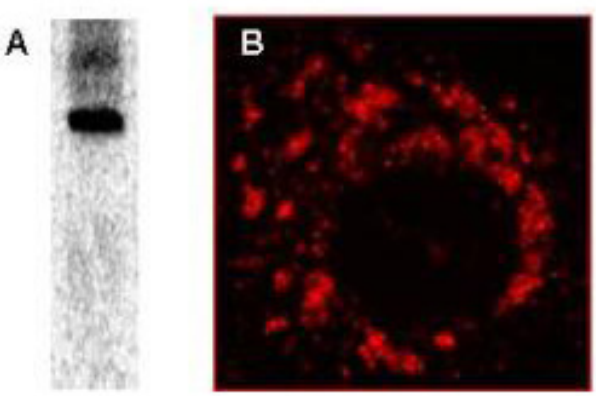
Images



Immunofluorescence Microscopy
Colocalization of nsp8 with other replicase proteins and dsRNA in SARS-CoV-2-infected cells. (A) A549-ACE2 cells were infected with SARS-CoV-2 (MOI = 2), fixed at 6-h postinfection (hpi), and costained with rabbit anti-SARS nsp8 and appropriate home-made mouse anti-SARS-CoV-2 nsp sera or mouse anti-dsRNA MAb, followed by staining with Alexa Fluor 488-conjugated goat anti-rabbit (green) and Alexa Fluor 555-conjugated goat anti-mouse (red). Cell nuclei were stained with DAPI (blue) and examined by confocal microscopy. Images in the fifth column were obtained at higher magnification to show single-cell details of fluorescence labeling; scale bars represent 10 μm. The intensity distribution describes the colocalization of nsp8 with other replicase proteins for specific fluorescence along the indicated line. (B) Pearson's correlation analysis demonstrated colocalization of nsp8 with other replicase proteins. One-way analysis of variance (ANOVA) was used for multiple comparisons on the colocalization between nsp8 and different nsps in GraphPad Prism 8.4.3 software. ****, $P < 0.0001$; ***, $P < 0.001$. Fig 7. PMID: 35730969



Immunofluorescence Microscopy
Time course of nsp8 and nsp13 detection. A549-ACE2 cells were mock infected or infected with SARS-CoV-2 (MOI 2), fixed at 2-, 3-, 4-, 6-, 8-, and 24-h postinfection (hpi), and costained with home-made mouse anti-SARS-CoV-2 N protein serum and rabbit anti-SARS nsp8 (A) or nsp13 (B) polyclonal, followed by staining with goat anti-mouse secondary antibody conjugated with Alexa Fluor 488 (green) and goat anti-rabbit secondary antibody conjugated with Alexa Fluor 555 (red). The cell nuclei were stained with DAPI (blue) and examined by confocal microscopy. No specific signal was observed at 2 and 3 hpi (not shown); scale bars represent 10 µm. The intensity distribution describes the timing of expression of nsp8 or nsp13 for specific fluorescence along the indicated line. (C) Pearson's correlation was used to analyze changes in nsp8 or nsp13 over time. One-way analysis of variance (ANOVA) was used for multiple comparisons between different times among the nsp8 or nsp13 in GraphPad Prism 8.4.3 software. ****, P < 0.0001. Fig 6. PMID: 35730969



Immunofluorescence Microscopy
Immunoprecipitation followed by western blotting using Rockland's 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.

References

- Shi FS et al. Expression Profile and Localization of SARS-CoV-2 Nonstructural Replicase Proteins in Infected Cells. *Microbiol Spectr.* (2022)

Disclaimer

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