abcam

ab283914

Anti-SARS-CoV2 orf8 antibody [EPR24851-65]

Recombinant | RabMAb | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR24851-65
Purification technique	Affinity purification Protein A
Concentration	1 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

ELISA

Tested

Species	SARS-CoV-2
Dilution info	0.00002-1 µg/mL
Notes	-

Not recommended

Species	Transfected cell line - SARS-CoV-2
Dilution info	-
Notes	-

WB

Tested

Species	SARS-CoV-2
Dilution info	1/1000
Notes	-

Not recommended

Species	Transfected cell line - SARS-CoV-2
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Transfected cell line - SARS-CoV-2
Dilution info	1/100
Notes	-

Expected

Species	SARS-CoV-2
Dilution info	Use at an assay dependent concentration.
Notes	-

Flow Cyt (Intra)

Tested

Species	Transfected cell line - SARS-CoV-2
Dilution info	1/500
Notes	-

Expected

Species	SARS-CoV-2
Dilution info	Use at an assay dependent concentration.
Notes	-

IHC-P

Tested

Species	SARS-CoV-2
Dilution info	1/100
Notes	-

Not recommended

Species	Transfected cell line - SARS-CoV-2
Dilution info	-
Notes	-

Target data

See full target information SARS-CoV2 orf8

Function

Plays a role in modulating the host immune response (PubMed:31986261, PubMed:35343786, PubMed:36689483). May act as a secreted virokine by mimicking interleukin-17A (IL17A), and thereby binding to the IL17RA receptor, leading to activation of the IL17 pathway and increased secretion of pro-inflammatory factors (PubMed:35343786, PubMed:36689483). Contributes to the cytokine storm during SARS-CoV-2 infection when secreted by unconventional pathway (PubMed:33723527, PubMed:36689483). May act by down-regulating major histocompability complex class I (MHC-I) at cell surface (PubMed:34021074, PubMed:35157849). May inhibit expression of some members of the IFN-stimulated gene (ISG) family including hosts IGF2BP1/ZBP1, MX1 and MX2, and DHX58 (PubMed:34177923).

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free batch production

For more information, read more on recombinant antibodies.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	SARS-CoV-2 Orf8 also known as open reading frame 8 is a protein of interest in studying the SARS-CoV-2 virus. It is a viral accessory protein that weighs around 22 kDa and is expressed abundantly in infected host cells especially within the endoplasmic reticulum. Researchers identify this protein as having a unique location among Coronaviridae setting it apart in function when compared to other viral proteins.
Biological function summary	The Orf8 protein interacts with host cellular machinery to affect immune response. It is not part of any known stable complexes but it modulates the unfolded protein response (UPR) in cells influencing how proteins fold and maintaining cellular homeostasis under stress conditions. Orf8 can also impact the major

	histocompatibility complex class I (MHC-I) pathway which has implications on immune evasion by the virus.
Pathways	Studies indicate that SARS-CoV-2 Orf8 plays roles in immune modulation and protein processing pathways. It interacts with the UPR pathway notably affecting the IRE1 signaling. Comparisons to other viral accessory proteins such as SARS-CoV-2 Orf3b reveal distinct impacts on host immune responses illustrating the diverse strategies the virus employs to enhance survival and replication.
Associated diseases and disorders	Orf8's expression links with COVID-19 severity contributing to immune dysregulation seen in patients. It has connections to inflammatory disorders due to its interference with the MHC-I pathway. Researchers study Orf8 alongside Nsp1 to understand the synergistic effects in manipulating host immune responses aiding in the pathogenesis of the disease. Understanding Orf8 can provide insights into targeted therapeutic interventions for COVID-19.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

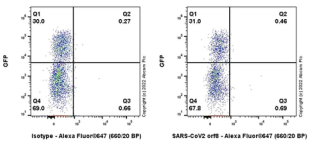
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

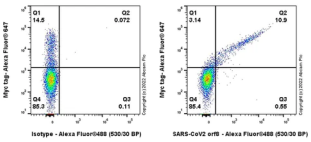
7 product images



Flow Cytometry (Intracellular) - Anti-SARS-CoV2 orf8 antibody [EPR24851-65] (ab283914)

SARS-CoV2 orf8 Flow Cytometry (Intracellular) staining using rabbit Anti-SARS-CoV2 orf8 antibody

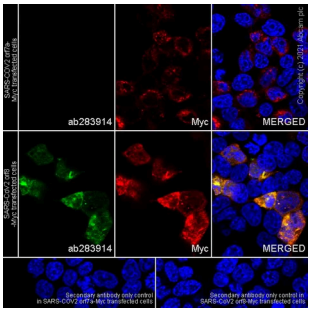
Intracellular Flow Cytometry analysis of HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) cells transfected with a SARS-COV2 orf7a expression vector containing a GFP tag labeling SARS-CoV2 orf8 with ab283914 at 1/500 dilution (right). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti-rabbit IgG (Alexa Fluor® 647, ab150083) secondary antibody was used at 1/5000 dilution. Isotype control - Rabbit monoclonal IgG (left) (ab172730). No cross-reactivity with SARS-COV2 orf7a.



Flow Cytometry (Intracellular) - Anti-SARS-CoV2 orf8 antibody [EPR24851-65] (ab283914)

SARS-CoV2 orf8 Flow Cytometry (Intracellular) staining using rabbit Anti-SARS-CoV2 orf8 antibody

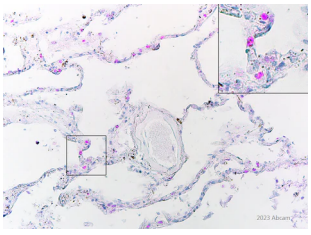
Intracellular Flow Cytometry analysis of HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) cells transfected with a SARS-CoV2 orf8 expression vector containing a myc tag labeling SARS-CoV2 orf8 with ab283914 at 1/500 dilution (right). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti-rabbit IgG (Alexa Fluor® 488, ab150081) secondary antibody was used at 1/5000 dilution. Isotype control - Rabbit monoclonal IgG (left) (ab172730).



Immunocytochemistry/ Immunofluorescence - Anti-SARS-CoV2 orf8 antibody [EPR24851-65] (ab283914)

SARS-CoV2 orf8 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-SARS-CoV2 orf8 antibody

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) cells labelling SARS-CoV2 orf8 with primary antibody anti-SARS-CoV2 orf8 (ab283914) at 1/100 dilution, followed by AlexaFluor®594 Goat anti-Rabbit (green) (ab150088) secondary antibody at 1/1000 dilution. Confocal image showing cytoplasmic and nuclear staining in HEK-293T cells transfected with a SARS-CoV2 orf8 expression vector containing a myc tag, no staining in HEK-293T cells transfected with a SARS-COV2 orf7a expression vector containing a myc tag. Myc-Tag Mouse mab (Alexa Fluor® 647) (red) was used to counterstain at 1/100 dilution. The nuclear counter stain is DAPI (blue).

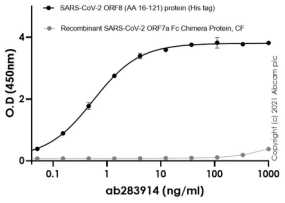


This image is courtesy of a customer review submitted by Dr, Aron Emmi

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SARS-CoV2 orf8 antibody [EPR24851-65] (ab283914)

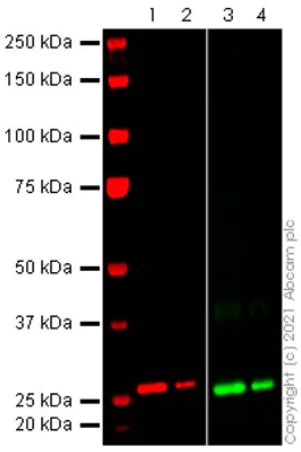
ab283914 staining SARS-CoV-2 nsp8 in Human Lung (SARS-CoV-2 RNA+) tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with formaldehyde, antigen retrieval was by heat mediation in a EDTA Buffer (pH 9). Samples were incubated with primary antibody (1/100) for 45 minutes at 24°C. A Rabbit HRP-conjugated secondary antibody was used.

ELISA - Anti-SARS-CoV2 orf8 antibody [EPR24851-65] (ab283914)



Indirect ELISA showing primary antibody ab283914 binding to SARS-CoV2 ORF8 (AA 16-121) protein (His tag). Plates were coated with SARS-CoV2 ORF8 (AA 16-121) protein (His tag) and Recombinant SARS-CoV2 ORF7a Fc Chimera protein at 1000 ng/ml. Binding of ab283914 was assessed in a serial dilution range 0.016- 1000 ng/mL (a 3-fold serial dilution).
Binding was detected using pre-adsorbed secondary antibody, goat anti-rabbit IgG H&L (HRP, ab97080) at 1/2000 dilution.

Western blot - Anti-SARS-CoV2 orf8 antibody [EPR24851-65] (ab283914)



Lanes 1 & 2: Red – loading control ab18184 (Mouse monoclonal [HIS.H8] to 6X His tag®) observed at 27 kDa.
Lanes 3 & 4: Green – ab283914 observed at 27 kDa

ab283914 was shown to bind specifically to SARS-CoV-2 ORF8 protein in Western blot. Samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) at 1/20000 dilution.

Blocking buffer: 3% milk in TBS-0.1% Tween® 20 (TBS-T)

All lanes:
Western blot - Anti-SARS-CoV2 orf8 antibody [EPR24851-65] (ab283914) at 1/1000 dilution

Lanes 1 and 3:
SARS-CoV-2 ORF8 (AA 16-121) protein (His tagged) at 0.5 µg

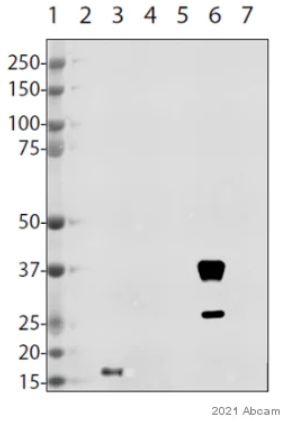
Lanes 2 and 4:
SARS-CoV-2 ORF8 (AA 16-121) protein (His tagged) at 0.2 µg

Secondary

All lanes:
Western blot - Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/20000 dilution

Observed band size: 27 kDa

Western blot - Anti-SARS-CoV2 orf8 antibody [EPR24851-65] (ab283914)



All lanes:
Western blot - Anti-SARS-CoV2 orf8 antibody [EPR24851-65] (ab283914) at 1 µg/mL

Lane 1:
Precision Plus Protein™ Dual Color Standard

Lane 2:
CaCo2 Uninfected whole cell lysate at 40 µg

Lane 3:
CaCo2 infected with SARS-CoV-2 whole cell lysate at 40 µg

This image is courtesy of a customer review submitted by Nicola Goodman

Lane 4:
CaCo2 infected with CoV-NL63 whole cell lysate at 40 µg

Lane 5:
CaCo2 infected with CoV-OC463 whole cell lysate at 40 µg

Lane 6:
recombinant SARS CoV-2 orf8 + GST tag 500ng at 500 ng

Lane 7:
A549 WT whole cell lysate at 15 µg

Secondary

All lanes:
LiCor goat anti-rabbit 800CW

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.