abcam

ab315964

Anti-SARS-CoV2 nsp12 antibody [EPR24837-213]

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	EPR24837-213
Purification technique	Affinity purification Protein A
Concentration	1 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

WB

Tested

Species	SARS-CoV-2
Dilution info	1/2000
Notes	-

ICC/IF

Tested

Species	SARS-CoV-2
Dilution info	1/500
Notes	-

IP

Tested

Species	SARS-CoV-2
Dilution info	1/30
Notes	-

IHC-P

Tested

Species	SARS-CoV-2
Dilution info	1/4000
Notes	-

Flow Cyt

Tested

Species	SARS-CoV-2
Dilution info	1/500
Notes	-

Target data

See full target information rep

Function Replicase polyprotein 1ab. Multifunctional protein involved in the transcription and replication of viral RNAs. Contains the proteinases responsible for the cleavages of the polyprotein. Host translation inhibitor nsp1. Inhibits host translation by associating with the open head conformation of the 40S subunit

(PubMed:32680882, PubMed:32908316, PubMed:33080218, PubMed:33479166). The C-terminus binds to and obstructs ribosomal mRNA entry tunnel (PubMed:32680882, PubMed:32908316, PubMed:33080218, PubMed:33479166). Thereby inhibits antiviral response triggered by innate immunity or interferons (PubMed:32680882, PubMed:32979938, PubMed:33080218). The nsp1-40S ribosome complex further induces an endonucleolytic cleavage near the 5'UTR of host mRNAs, targeting them for degradation (By similarity). This inhibits the integrated stress response (ISR) in the infected cell by preventing EIF2S1/eIF2-alpha phosphorylation upstream of stress granule formation and depletes host G3BP1 (PubMed:36534661). Viral mRNAs less susceptible to nsp1-mediated inhibition of translation, because of their 5'-end leader sequence (PubMed:32908316, PubMed:33080218). Non-structural protein 2. Enhances mRNA repression of the 4EHP-GYF2 complex in the host, thereby inhibiting the antiviral response and facilitating SARS-CoV-2 replication. Possibly acts in cooperation with nsp1, which induces ribosome stalling on host mRNA, triggering mRNA repression by the host 4EHP-GYF2 complex which is enhanced by nsp2. Papain-like protease nsp3. Responsible for the cleavages located at the N-terminus of the replicase polyprotein. Participates together with nsp4 in the assembly of virally-induced cytoplasmic double-membrane vesicles necessary for viral replication (PubMed:35551511). Antagonizes innate immune induction of type I interferon by blocking the phosphorylation, dimerization and subsequent nuclear translocation of host IRF3 (PubMed:32733001). Also prevents host NF-kappa-B signaling (By similarity). In addition, PL-PRO possesses a deubiquitinating/deISGylating activity and processes both 'Lys-48'- and 'Lys-63'-linked polyubiquitin chains from cellular substrates (PubMed:32726803). Cleaves preferentially ISG15 from antiviral protein IFIH1 (MDA5), but not RIGI (PubMed:33727702). Can play a role in host ADP-ribosylation by ADP-ribose (PubMed:32578982). Plays a role in the formation and maintenance of double membrane vesicles (DMVs) replication organelles (PubMed:35551511). DMVs are formed by nsp3 and nsp4, while nsp6 zippers ER membranes and connects to lipid droplets (PubMed:35551511). Non-structural protein 4. Plays a role in the formation and maintenance of double membrane vesicles (DMVs) replication organelles (PubMed:35551511). DMVs are formed by nsp3 and nsp4, while nsp6 zippers ER membranes and connects to lipid droplets (PubMed:35551511). 3C-like proteinase nsp5. Cleaves the C-terminus of replicase polyprotein at 11 sites (PubMed:32321856). Recognizes substrates containing the core sequence [ILMVF]-Q-|-[SGACN] (PubMed:32198291, PubMed:32272481). Cleaves and inactivates human TRMT1, preventing tRNA guanine(26)-dimethylation of tRNAs (PubMed:37073102, PubMed:38814682, PubMed:39773525). May cleave human NLRP1 in lung epithelial cells, thereby activating the NLRP1 inflammasome pathway (PubMed:35594856). May cleave human GSDMD, triggering alternative GSDME-mediated epithelial cell death upon activation of the NLRP1 inflammasome, which may enhance the release interleukins 1B, 6, 16 and 18 (PubMed:35594856). Also able to bind an ADP-ribose-1''-phosphate (ADRP) (PubMed:32198291, PubMed:32272481). Non-structural protein 6. Plays a role in the formation and maintenance of double membrane vesicles (DMVs) replication organelles (PubMed:35551511). DMVs are formed by nsp3 and nsp4, while nsp6 zippers ER membranes and connects to lipid droplets (PubMed:35551511). LDs are consumed during DMV formation (PubMed:35551511). Binds to host TBK1 without affecting TBK1 phosphorylation; the interaction with TBK1 decreases IRF3 phosphorylation, which leads to reduced IFN-beta production (PubMed:32979938). Non-structural protein 7. Plays a role in viral RNA synthesis (PubMed:32277040, PubMed:32358203, PubMed:32438371, PubMed:32526208). Forms a hexadecamer with nsp8 (8 subunits of each) that may participate in viral replication by acting as a primase. Alternatively, may synthesize substantially longer products than

oligonucleotide primers (By similarity). Non-structural protein 8. Plays a role in viral RNA synthesis (PubMed:32277040, PubMed:32358203, PubMed:32438371, PubMed:32526208). Forms a hexadecamer with nsp7 (8 subunits of each) that may participate in viral replication by acting as a primase. Alternatively, may synthesize substantially longer products than oligonucleotide primers (By similarity). Interacts with ribosome signal recognition particle RNA (SRP) (PubMed:33080218). Together with NSP9, suppress protein integration into the cell membrane, thereby disrupting host immune defenses (PubMed:33080218). Viral protein genome-linked nsp9. Forms a primer, NSP9-pU, which is utilized by the polymerase for the initiation of RNA chains (PubMed:37794589). Interacts with ribosome signal recognition particle RNA (SRP) (PubMed:33080218). Together with NSP8, suppress protein integration into the cell membrane, thereby disrupting host immune defenses (PubMed:33080218). Non-structural protein 10. Plays a pivotal role in viral transcription by stimulating both nsp14 3'-5' exoribonuclease (By similarity) and nsp16 2'-O-methyltransferase activities (PubMed:35944563). Therefore plays an essential role in viral mRNAs cap methylation. RNA-directed RNA polymerase nsp12. RNA-directed RNA polymerase that catalyzes the transcription of viral genomic and subgenomic RNAs. Acts in complex with nsp7 and nsp8 to transcribe both the minus and positive strands of genomic RNA (PubMed:32277040, PubMed:32358203, PubMed:32438371, PubMed:32526208). The kinase-like NiRAN domain of NSP12 attaches one or more nucleotides to the amino terminus of NSP9, forming a covalent RNA-protein intermediate that serves as transcription/replication primer (PubMed:37794589). Subgenomic RNAs (sgRNAs) are formed by discontinuous transcription: The polymerase has the ability to pause at transcription-regulating sequences (TRS) and jump to the leader TRS, resulting in a major deletion (PubMed:35706445). This creates a series of subgenomic RNAs that are replicated, transcribed and translated (PubMed:35706445). In addition, Nsp12 is a subunit of the viral RNA capping enzyme that catalyzes the RNA guanylyltransferase reaction for genomic and sub-genomic RNAs (PubMed:35944563). Subsequently, the NiRAN domain transfers RNA to GDP, and forms the core cap structure GpppA-RNA (PubMed:35944563). Helicase nsp13. Plays a role in viral RNA synthesis (PubMed:33232691). Multi-functional protein with a zinc-binding domain in N-terminus displaying RNA and DNA duplex-unwinding activities with 5' to 3' polarity. Activity of helicase is dependent on magnesium (By similarity). Binds to host TBK1 and inhibits TBK1 phosphorylation; the interaction with TBK1 decreases IRF3 phosphorylation, which leads to reduced IFN-beta production (PubMed:32979938). Guanine-N7 methyltransferase nsp14. Plays a role in viral RNA synthesis through two distinct activities. The N7-guanine methyltransferase activity plays a role in the formation of the cap structure GpppA-RNA (PubMed:35944563). The proofreading exoribonuclease reduces the sensitivity of the virus to RNA mutagens during replication (By similarity). This activity acts on both ssRNA and dsRNA in a 3'-5' direction (By similarity). Uridylate-specific endoribonuclease nsp15. Plays a role in viral transcription/replication and prevents the simultaneous activation of host cell dsRNA sensors, such as MDA5/IFIH1, OAS, and PKR (By similarity). Acts by degrading the 5'-polyuridines generated during replication of the poly(A) region of viral genomic and subgenomic RNAs (PubMed:33504779, PubMed:33564093). Catalyzes a two-step reaction in which a 2'3'-cyclic phosphate (2'3'-cP) is first generated by 2'-O transesterification, which is then hydrolyzed to a 3'-phosphate (3'-P) (PubMed:33504779, PubMed:33564093). If not degraded, poly(U) RNA would hybridize with poly(A) RNA tails and activate host dsRNA sensors (By similarity). May bind genomic dsRNA in association with the replication-transcription complex (RTC), and play a role in nsp12 discontinous transcription (PubMed:34562452, PubMed:35706445). 2'-O-methyltransferase nsp16. Methyltransferase that

mediates mRNA cap 2'-O-ribose methylation to the 5'-cap structure of viral mRNAs (PubMed:35944563). N7-methyl guanosine cap is a prerequisite for binding of nsp16 (PubMed:35944563). Therefore, it plays an essential role in cap methylation of viral mRNAs, which is essential to evade the immune system, especially when restricted by human IFIT1 and IFIT3 (PubMed:35944563, PubMed:36722972, PubMed:36285486). May disrupt host mRNA splicing in nucleus by interacting with pre-mRNA Recognition Domains of the U1 and U2 snRNAs (PubMed:33080218).

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-CoV2 nsp12 also known as RNA-dependent RNA polymerase (RdRp) plays a central role in the viral replication cycle of the SARS-CoV2 virus. This enzyme has a molecular mass of approximately 106 kDa. It carries out its activities inside the

	host cell where the virus replicates such as in lung epithelial cells. Mechanically nsp12 facilitates the production of new viral RNA strands by copying the viral RNA genome allowing the virus to proliferate within the host.
Biological function summary	RNA-dependent RNA polymerase coordinates with other non-structural proteins to form the replication-transcription complex. Within this complex vital functions include the catalysis of viral RNA synthesis. The enzyme interacts with cofactors like nsp7 and nsp8 to enhance its polymerase activity and ensure efficient replication of viral RNA. These interactions are important to the viral life cycle making nsp12 an important player in SARS-CoV2 biology.
Pathways	The replication and transcription activities mediated by nsp12 integrate into the viral RNA synthesis pathway. This pathway is essential for the synthesis of subgenomic RNA and ultimately the production of structural proteins necessary for virion assembly. Nsp12 works closely with proteins such as nsp7 and nsp8 forming a replication complex to execute these functions. These interactions ensure the processive synthesis of RNA and are highly conserved across coronaviruses.
Associated diseases and disorders	Nsp12 closely associates with COVID-19 the disease caused by SARS-CoV2. This protein's role in viral replication makes it a target for antiviral drug development as inhibiting nsp12 could block viral replication and reduce viral load in infected individuals. Remdesivir an antiviral medication targets this protein to impair its function. Mutation or dysfunction in nsp12 combined with its interaction with other viral proteins directly correlates with viral pathogenicity and disease severity highlighting its significance in the context of SARS-CoV2 infection.

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

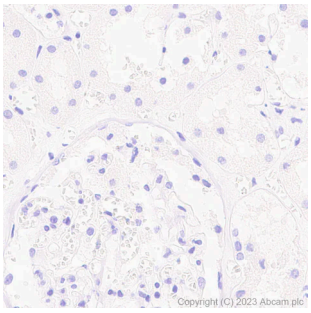
We do not recommend this combination. It is not covered by our product promise.

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.

9 product images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SARS-CoV2 nsp12 antibody [EPR24837-213] (ab315964)

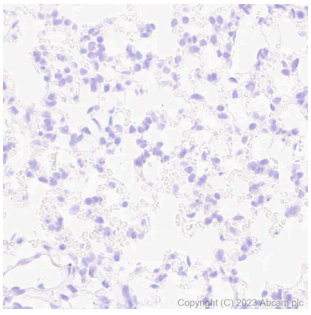
SARS-CoV2 nsp12 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human kidney tissues using rabbit Anti-SARS-CoV2 nsp12 antibody

Immunohistochemical analysis of paraffin-embedded human kidney tissues labelling SARS-CoV2 nsp12 with ab315964 at a 1/4000 dilution, followed by LeicaDS9800 (Bond™ Polymer Refine Detection) ready to use secondary antibody. Counter stained with Hematoxylin.

Negative control: no staining on human kidney. The section was incubated with ab315964 for 30 mins at room temperature.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SARS-CoV2 nsp12 antibody [EPR24837-213] (ab315964)

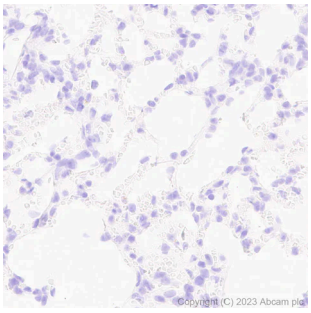
SARS-CoV2 nsp12 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Mouse tissues using rabbit Anti-SARS-CoV2 nsp12 antibody

Immunohistochemical analysis of paraffin-embedded mouse lung tissues labelling SARS-CoV2 nsp12 with ab315964 at a 1/4000 dilution, followed by LeicaDS9800 (Bond™ Polymer Refine Detection) ready to use secondary antibody. Counter stained with Hematoxylin.

Negative control: no staining on mouse lung. The section was incubated with ab315964 for 30 mins at room temperature.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SARS-CoV2 nsp12 antibody [EPR24837-213] (ab315964)

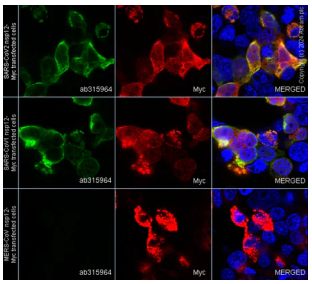
SARS-CoV2 nsp12 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Rat lung tissues using rabbit Anti-SARS-CoV2 nsp12 antibody

Immunohistochemical analysis of paraffin-embedded rat lung tissues labelling SARS-CoV2 nsp12 with ab315964 at 1/4000 dilution, followed by LeicaDS9800 (Bond™ Polymer Refine

Detection) ready to use secondary antibody. Counter stained with Hematoxylin.

Negative control: no staining on rat lung. The section was incubated with ab315964 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins.

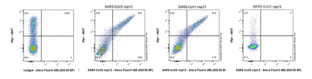


Immunocytochemistry/ Immunofluorescence - Anti-SARS-CoV2 nsp12 antibody [EPR24837-213] (ab315964)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized 293T (human embryonic kidney epithelial) cells labelling SARS-CoV2 nsp12 with ab315964 at a 1/500 dilution, followed by ab150081 AlexaFluor® 488 Goat anti-Rabbit IgG H&L secondary antibody at a 1/1000 dilution. Myc-Tag Mouse mAb (Alexa Fluor® 647) at a 1/100 dilution used as a counterstain. The Nuclear counterstain was DAPI (blue).

Confocal image showing positive staining in 293T cells transfected with a SARS-CoV2 nsp12 expression vector containing a myc-His-tag® and 293T cells transfected with a SARS-CoV1 nsp12 expression vector containing a myc-His-tag® (shown in green). In addition, it shows no staining in 293T cells transfected with a MERS-CoV2 nsp12 expression vector containing a myc-His-tag®.

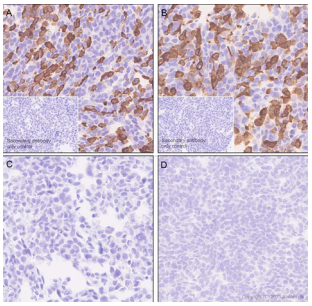
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Flow Cytometry - Anti-SARS-CoV2 nsp12 antibody [EPR24837-213] (ab315964)

Flow cytometric analysis of permeabilized with 90% methanol and 4% paraformaldehyde fixed HEK-293T cells:
1.Isotype
2.Transfected with a SARS-CoV2 nsp12 expression vector containing a myc- tag
3.Transfected with a SARS-CoV1 nsp12 expression vector containing a myc- tag
4.Transfected with a MERS-CoV2 nsp12 expression vector

Labelling SARS-CoV2 nsp12 with ab315964 at a 1/500 dilution (red line) compared with a Rabbit monoclonal IgG (ab172730) Isotype control (black line) and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (blue line). Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at a 1/5000 dilution was used as the secondary antibody.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SARS-CoV2 nsp12 antibody [EPR24837-213] (ab315964)

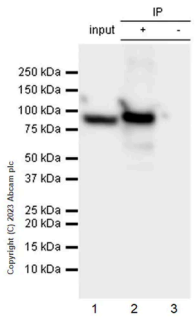
Immunohistochemical analysis of paraffin-embedded sections:
(A) HEK-293T (human embryonic kidney epithelial cell line) transfected with a SARS-CoV2 nsp12 expression vector containing a his tag.
(B) HEK-293T transfected with a SARS-CoV1 nsp12 expression vector containing a his tag.
(C) HEK-293T transfected with a MERS-CoV nsp12 expression vector containing a his tag.
(D) HEK-293T transfected with empty vector containing a his tag.

SARS-CoV2 nsp12 was labeled with ab315964 at a 1/4000 dilution, followed by LeicaDS9800 (Bond™ Polymer Refine Detection) ready to use secondary antibody. Counter stained with Hematoxylin.

Positive staining on- (A) HEK-293Ttransfected with a SARS-CoV2 nsp12 expression vector containing a his tag and (B) HEK-293T transfected with a SARS-CoV1 nsp12 expression vector containing a his tag. No staining on (C) HEK-293T transfected with a MERS-CoV nsp12 expression vector containing a his tag and (D) HEK-293T transfected with empty vector containing a his tag. The section was incubated with ab315964 for 30 mins at room temperature.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins.



Immunoprecipitation - Anti-SARS-CoV2 nsp12 antibody [EPR24837-213] (ab315964)

SARS-CoV2 nsp12 was immunoprecipitated from 0.35 mg HEK-293 transfected with SARS-CoV2 nsp12 (aa4393-5324) expression vector containing a myc-His-tag®, whole cell lysate with ab315964 at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab315964 at 1/1000 dilution. VeriBlot for IP secondary antibody (HRP) (ab131366) was used at 1/5000 dilution.

Lane 1(Input): HEK-293 transfected with SARS-CoV2 nsp12 (aa4393-5324) expression vector containing a myc-His-tag®, whole cell lysate 10 µg
Lane 2(+): HEK-293 transfected with SARS-CoV2 nsp12 (aa4393-5324) expression vector containing a myc-His-tag®, whole cell lysate.
Lane 3(-): Rabbit monoclonal IgG (ab172730) instead of ab315964 in HEK-293 transfected with SARS-CoV2 nsp12 (aa4393-5324) expression vector containing a myc-His-tag®, whole cell lysate.
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

All lanes:
Immunoprecipitation - Anti-SARS-CoV2 nsp12 antibody [EPR24837-213] (ab315964) at 1/30 dilution

Lane 1:
HEK-293 transfected with SARS-CoV2 nsp12 (aa4393-5324) expression vector containing a myc-His-tag®, whole cell lysate at 10 µg

Lane 2:
HEK-293 transfected with SARS-CoV2 nsp12 (aa4393-5324) expression vector containing a myc-His-tag®, whole cell lysate.

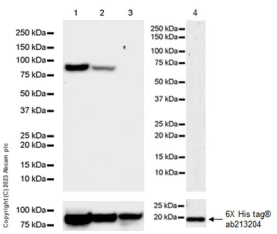
Lane 3:
Rabbit monoclonal IgG (ab172730) instead of ab315964 in HEK-293 transfected with SARS-CoV2 nsp12 (aa4393-5324) expression vector containing a myc-His-tag®, whole cell lysate

Secondary

All lanes:
Immunoprecipitation - VeriBlot for IP Detection Reagent (HRP) (ab131366) at 1/5000 dilution

Observed band size: 90 kDa

Exposure time: 6s



Western blot - Anti-SARS-CoV2 nsp12 antibody [EPR24837-213] (ab315964)

Blocking and dilution buffer:5% NFDM/TBST.
This antibody does not cross-react with MERS-CoV nsp12 and SARS-CoV2 nsp1, but show cross-reactivity with SARS-CoV1 nsp12.

In western blot, anti-His antibody (ab213204) staining at a 1/5000 dilution.

All lanes:
Western blot - Anti-SARS-CoV2 nsp12 antibody [EPR24837-213] (ab315964) at 1/1000 dilution

Lane 1:
HEK-293 transfected with SARS-CoV2 nsp12 (aa4393-5324) expression vector containing a myc-His-tag®, whole cell lysate at 20 µg

Lane 2:
HEK-293 transfected with SARS-CoV1 nsp12 (aa4370-5301) expression vector containing a myc-His-tag®, whole cell lysate at 20 µg

Lane 3:
HEK-293 transfected with MERS-CoV nsp12 (aa4378-5301) expression vector containing a myc-His-tag®, whole cell lysate at 20 µg

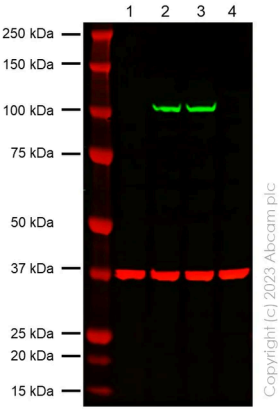
Lane 4:
His-tagged SARS-CoV2 nsp1 (aa1-180) recombinant protein at 10 ng

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Observed band size: 90 kDa

Exposure time: 48s



Western blot - Anti-SARS-CoV2 nsp12 antibody [EPR24837-213] (ab315964)

This antibody does not cross-react with MERS-CoV nsp12.

Merged signal (red and green). Green - ab315964 observed at 107 kDa. Red - loading control, ab8245, observed at 37 kDa.

All lanes:
Western blot - Anti-SARS-CoV2 nsp12 antibody [EPR24837-213] (ab315964) at 1/2000 dilution

Lane 1:
HEK-293 cells transfected with control expression vector, whole cell lysate at 20 µg

Lane 2:
HEK-293 transfected with SARS-CoV2 nsp12 expression vector, whole cell lysate at 20 µg

Lane 3:
HEK-293 transfected with SARS-CoV1 nsp12 expression vector, whole cell lysate at 20 µg

Lane 4:
HEK-293 transfected with MERS-CoV nsp12 expression vector, whole cell lysate at 20 µg

Secondary

All lanes:
Goat anti-Rabbit IgG H&L (800CW) preadsorbed, green channel at 1/20000 dilution

Observed band size: 107 kDa, 37 kDa

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