abcam

Product datasheet

Anti-CRISPR-Cas9 antibody [EPR18991] ab189380



★★★★★ 1 Abreviews 7 References 10 Images

Overview

Product name Anti-CRISPR-Cas9 antibody [EPR18991]

Description Rabbit monoclonal [EPR18991] to CRISPR-Cas9

Host species Rabbit

Tested applications Suitable for: ICC, IHC-P, ICC/IF, WB, Flow Cyt (Intra)

Species reactivity

Predicted to work with: Streptococcus pyogenes

Recombinant fragment. This information is proprietary to Abcam and/or its suppliers. **Immunogen**

WB and Flow Cyt (intra): HEK-293 whole cell lysate transfected with CRISPR-Cas9 (Q99ZW2, Positive control

> Streptococcus pyogenes serotype M1) with GFP-Myc tag. IHC: 293T cells transfected with Streptococcus pyogenes serotype M1 Cas9 (pcDNA3.1(+)-GFP-Myc). ICC/IF: 293T cells

transfected with CRISPR-Cas9 with GFP-tag.

General notes 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 production

For more information see here.

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

Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long

term. Avoid freeze / thaw cycle.

Storage buffer

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

Purity Protein A purified

Clonality Monoclonal

Clone number

EPR18991

Isotype

ΙgG

Applications

The Abpromise guarantee

Our Abpromise quarantee covers the use of ab189380 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

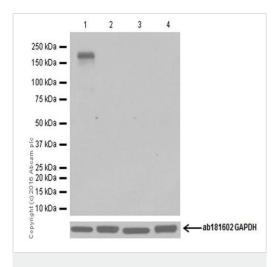
Application	Abreviews	Notes
ICC		1/100.
IHC-P		1/100. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		1/500.
WB	★★★★ ★ (1)	1/20000. Detects a band of approximately 184 kDa (predicted molecular weight: 158 kDa).
Flow Cyt (Intra)		1/70.

Target

Relevance

[FUNCTION] CRISPR (clustered regularly interspaced short palindromic repeat) is an adaptive immune system that provides protection against mobile genetic elements (viruses, transposable elements and conjugative plasmids). CRISPR clusters contain spacers, sequences complementary to antecedent mobile elements, and target invading nucleic acids. CRISPR clusters are transcribed and processed into CRISPR RNA (crRNA) (Probable). In type II CRISPR systems correct processing of pre-crRNA requires a trans-encoded small RNA (tracrRNA), endogenous ribonuclease 3 (rnc) and this protein. The tracrRNA serves as a guide for ribonuclease 3-aided processing of pre-crRNA. Subsequently Cas9/crRNA/tracrRNA endonucleolytically cleaves linear or circular dsDNA target complementary to the spacer. The target strand not complementary to crRNA is first cut endonucleolytically, then trimmed by 3'-5' exonucleolytically. DNA-binding requires protein and both RNA species. Cas9 probably recognizes a short motif in the CRISPR repeat sequences (the PAM or protospacer adjacent motif) to help distinguish self versus nonself.

Images



Western blot - Anti-CRISPR-Cas9 antibody [EPR18991] (ab189380)

All lanes : Anti-CRISPR-Cas9 antibody [EPR18991] (ab189380) at 1/20000 dilution

Lane 1: HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate transfected with CRISPR-Cas9 (Q99ZW2, Streptococcus pyogenes serotype M1) with GFP-Myc tag

Lane 2: HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

Lane 3 : NIH/3T3 (Mouse embryonic fibroblast cell line) whole cell lysate

Lane 4: Rat embryo lysate

Lysates/proteins at 20 µg per lane.

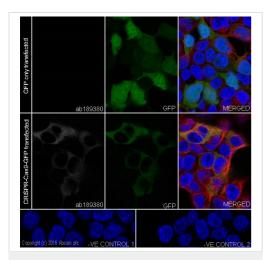
Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

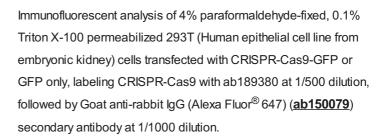
Predicted band size: 158 kDa **Observed band size:** 184 kDa

Exposure time: 10 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.



Immunocytochemistry/ Immunofluorescence - Anti-CRISPR-Cas9 antibody [EPR18991] (ab189380)



Confocal image showing positive staining on 293T cells transfected with CRISPR-Cas9 with GFP-tag.

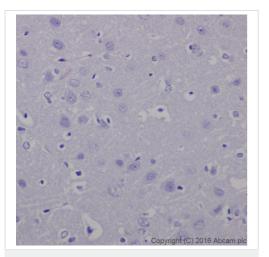
The nuclear counterstain is DAPI (blue).

Tubulin is detected with <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/1000 dilution and <u>ab150120</u> (Alexa Fluor[®] 594 Goat anti-Mouse secondary) at 1/1000 dilution (red).

The negative controls are as follows:-

-ve control 1: ab189380 at 1/500 dilution followed by **ab150120** (Alexa Fluor[®] 594 Goat anti-Mouse secondary) at 1/1000 dilution.

-ve control 2: <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/1000 dilution followed by <u>ab150079</u> (Alexa Fluor[®] 647 Goat Anti-Rabbit lgG H&L) at 1/1000 dilution.



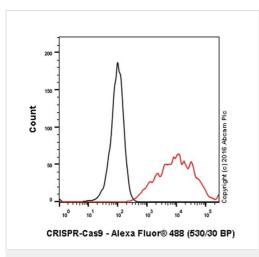
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CRISPR-Cas9 antibody [EPR18991] (ab189380)

Immunohistochemical analysis of paraffin-embedded Rat cerebrum tissue labeling CRISPR-Cas9 with ab189380 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

No staining on rat cerebrum is observed.

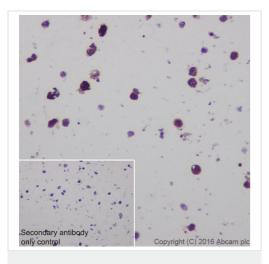
Counter stained with Hematoxylin.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Flow Cytometry (Intracellular) - Anti-CRISPR-Cas9 antibody [EPR18991] (ab189380)

Intracellular Flow Cytometry analysis of HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate (transfected with CRISPR-Cas9 (Q99ZW2, Streptococcus pyogenes serotype M1) with GFP-Myc tag) labelling CRISPR-Cas9 (red) with ab189380 at dilution of 1/70. The secondary antibody used was Alexa Fluorr® 488 goat-anti-rabbit lgG (1/2000). Cells were fixed with 4% paraformaldehyde. Isotype control antibody was (ab172730) Rabbit monoclonal lgG (black).



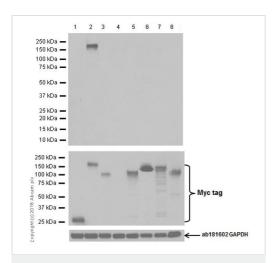
Immunocytochemistry - Anti-CRISPR-Cas9 antibody [EPR18991] (ab189380)

Immunocytochemical analysis of agarose-embedded sections of 293T (Human epithelial cell line from embryonic kidney) cells transfected with *Streptococcus pyogenes serotype M1* Cas9 (pcDNA3.1(+)-GFP-Myc) labeling CRISPR-Cas9 with ab189380 at 1/100 dilution, followed by Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

Positive staining on 293T cells transfected with *Streptococcus pyogenes serotype M1* Cas9 (pcDNA3.1(+)-GFP-Myc) is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.



Western blot - Anti-CRISPR-Cas9 antibody [EPR18991] (ab189380)

All lanes : Anti-CRISPR-Cas9 antibody [EPR18991] (ab189380) at 1/20000 dilution

Lane 1 : Empty vector with GFP-Myc tag (vector control) transfected HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate

Lane 2: HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate transfected with CRISPR-Cas9 (Q99ZW2, Streptococcus pyogenes serotype M1) with GFP-Myc tag

Lane 3: HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate transfected with CRISPR-Cas9 (G3ECR1, Streptococcus thermophilus, N-terminal aa1-800) with GFP-Myc tag

Lane 4 : Empty vector with Myc-His tag (vector control) transfected HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate

Lane 5 : HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate transfected with CRISPR-Cas9 (A1IQ68, Neisseria meningitidis serogroup A / serotype 4A (strain Z2491)) with Myc-His tag

Lane 6: HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate transfected with CRISPR-Cas9 (G3ECR1, Streptococcus thermophilus) with Myc-His tag

Lane 7: HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate transfected with CRISPR-Cas9 (Q03JI6, Streptococcus thermophilus (strain ATCC BAA-491 / LMD-9)) with Myc-His tag

Lane 8: HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate transfected with CRISPR-Cas9 (J7RUA5, Staphylococcus aureus subsp. aureus) with Myc-His tag

Lysates/proteins at 20 µg per lane.

Secondary

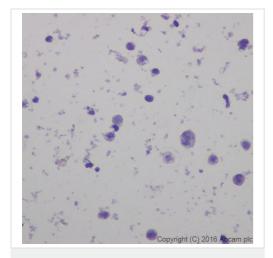
All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

Predicted band size: 158 kDa

Observed band size: 184 kDa

Exposure time: 3 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

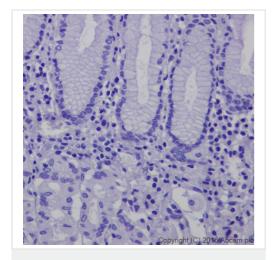


Immunocytochemistry - Anti-CRISPR-Cas9 antibody [EPR18991] (ab189380)

Immunocytochemical analysis of agarose-embedded sections of 293T (Human epithelial cell line from embryonic kidney) cells transfected with blank pcDNA3.1(+)-GFP-Myc vector labeling CRISPR-Cas9 with ab189380 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

Negative on 293T cells transfected with blank pcDNA3.1(+)-GFP-Myc vector.

Counter stained with Hematoxylin.



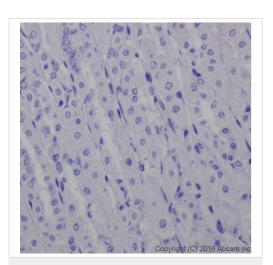
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CRISPR-Cas9 antibody [EPR18991] (ab189380)

Immunohistochemical analysis of paraffin-embedded Human stomach tissue labeling CRISPR-Cas9 with ab189380 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

No staining on Human stomach is observed.

Counter stained with Hematoxylin.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



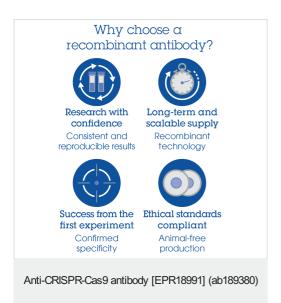
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CRISPR-Cas9 antibody [EPR18991] (ab189380)

Immunohistochemical analysis of paraffin-embedded Mouse stomach tissue labeling CRISPR-Cas9 with ab189380 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

No staining on mouse stomach is observed.

Counter stained with Hematoxylin.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



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