abcam

Product datasheet

Anti-CRISPR-Cas9 antibody [EPR19799] ab203933

Recombinant RabMAb

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Overview

Product name	Anti-CRISPR-Cas9 antibody [EPR19799]
Description	Rabbit monoclonal [EPR19799] to CRISPR-Cas9
Host species	Rabbit
Tested applications	**Suitable for:** ICC, WB, ICC/IF, IP, Flow Cyt (Intra), IHC-P
Species reactivity	**Predicted to work with:** Staphylococcus aureus
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HEK-293 whole cell lysate transfected with CRISPR-Cas9 (J7RUA5, S.aureus) with Myc-His tag. ICC: HeLa cells transfected with S.aureus Cas9 (pcDNA3.1(+)-Myc-His). ICC/IF: 293T cells transfected with CRISPR-Cas9 (J7RUA5, S.aureus) with Myc-His tag. Flow Cyt (intra): HEK-293 whole cell lysate transfected with CRISPR-Cas9 (J7RUA5, S.aureus) with Myc-His tag. IP: HEK-293 whole cell lysate transfected with CRISPR-Cas9 (J7RUA5, S.aureus) with Myc-His 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	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EPR19799
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab203933 in the following tested applications.

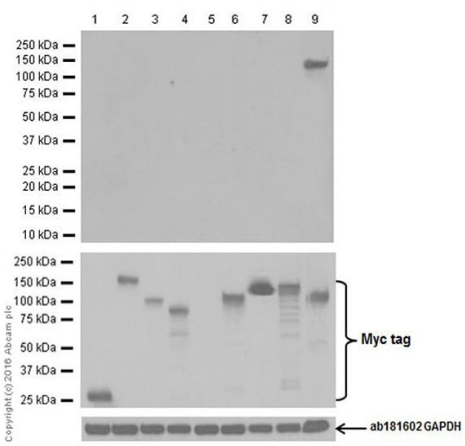
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC		1/500.
WB		1/20000. Detects a band of approximately 124 kDa (predicted molecular weight: 124 kDa).
ICC/IF		1/500.
IP		1/30.
Flow Cyt (Intra)		1/60.
IHC-P		1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

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 [EPR19799] (ab203933)

All lanes : Anti-CRISPR-Cas9 antibody [EPR19799] (ab203933) 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 : HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate transfected with CRISPR-Cas9 (G3ECR1, Streptococcus thermophilus, C–terminal aa801-1409) with GFP-Myc tag
Lane 5 : Empty vector with Myc-His tag (vector control) transfected HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate
Lane 6 : 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 7 : HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate transfected with CRISPR-Cas9 (G3ECR1, Streptococcus thermophilus) with Myc-His tag
Lane 8 : 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 9 : 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

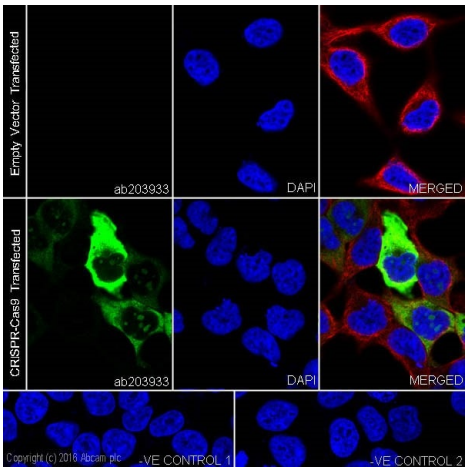
Lysates/proteins at 20 µg per lane.

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

Predicted band size: 124 kDa
Observed band size: 124 kDa

Exposure time: 1 second

Blocking/Dilution buffer: 5% NFDM/TBST.



Immunocytochemistry/ Immunofluorescence - Anti-CRISPR-Cas9 antibody [EPR19799] (ab203933)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized 293T (Human epithelial cell line from embryonic kidney) cells transfected with CRISPR-Cas9 (*J7RUA5, Staphylococcus aureus subsp. aureus*) with Myc-His tag or Empty vector, labeling CRISPR-Cas9 with ab203933 at 1/500 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution.

Confocal image showing positive staining on 293T cells transfected with CRISPR-Cas9 (*J7RUA5, Staphylococcus aureus subsp. aureus*) with Myc-His tag.

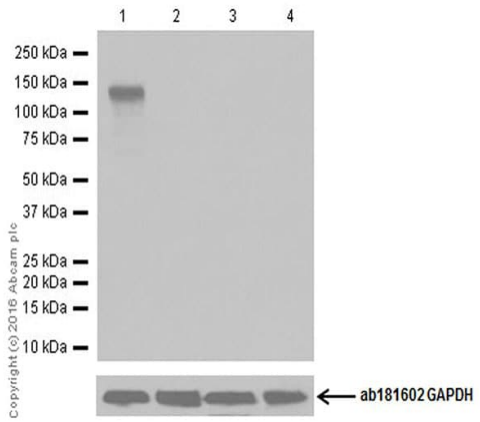
The nuclear counterstain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) at 1/1000 dilution and Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) preadsorbed (**ab150120**) at 1/1000 dilution (red).

The negative controls are as follows:-

-ve control 1: ab203933 at 1/500 dilution followed by **ab150120** at 1/1000 dilution.

-ve control 2: **ab7291** at 1/1000 dilution followed by **ab150077** at 1/1000 dilution.



Western blot - Anti-CRISPR-Cas9 antibody [EPR19799] (ab203933)

All lanes : Anti-CRISPR-Cas9 antibody [EPR19799] (ab203933) at 1/20000 dilution

Lane 1 : 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
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

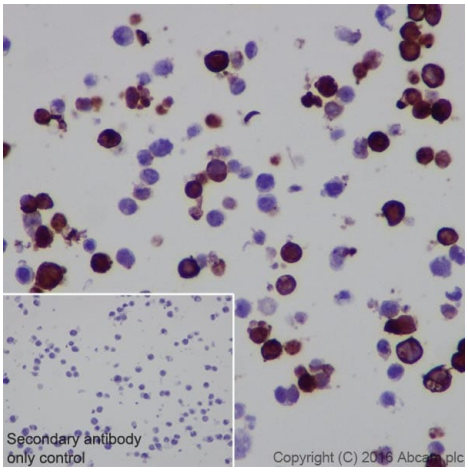
Lysates/proteins at 20 µg per lane.

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

Predicted band size: 124 kDa
Observed band size: 124 kDa

Exposure time: 5 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.



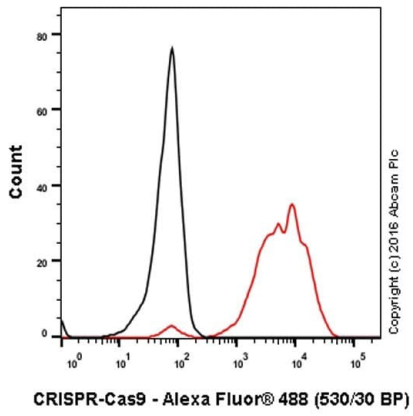
Immunocytochemistry - Anti-CRISPR-Cas9 antibody [EPR19799] (ab203933)

Immunocytochemical analysis of agarose-embedded 293T (Human epithelial cell line from embryonic kidney) cells transfected with *Staphylococcus aureus subsp. Aureus* Cas9 (pcDNA3.1(+)-Myc-His) labeling CRISPR-Cas9 with ab203933 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

Positive staining on 293T cells transfected with *Staphylococcus aureus subsp. Aureus* Cas9 (pcDNA3.1(+)-Myc-His) is observed.

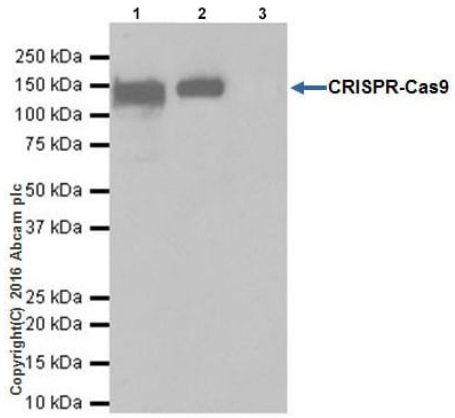
Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is **ab97051** at 1/500 dilution.



Flow Cytometry (Intracellular) - Anti-CRISPR-Cas9 antibody [EPR19799] (ab203933)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed HEK-293 (Human epithelial cell line from embryonic kidney) cells transfected withCRISPR-Cas9 (J7RUA5, Staphylococcus aureus subsp. aureus) with Myc-His tag labeling CRISPR-Cas9 with ab203933 at 1/60 dilution (red) compared with a Rabbit IgG,monoclonal [EPR25A] -Isotype control (**ab172730**) (black). Goat anti Rabbit IgG (Alexa Fluorr® 488) at 1/2000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-CRISPR-Cas9 antibody [EPR19799] (ab203933)

CRISPR-Cas9 was immunoprecipitated from 0.35 mg of 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 with ab203933 at 1/30 dilution.

Western blot was performed from the immunoprecipitate using ab203933 at 1/1000 dilution.

VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/10000 dilution.

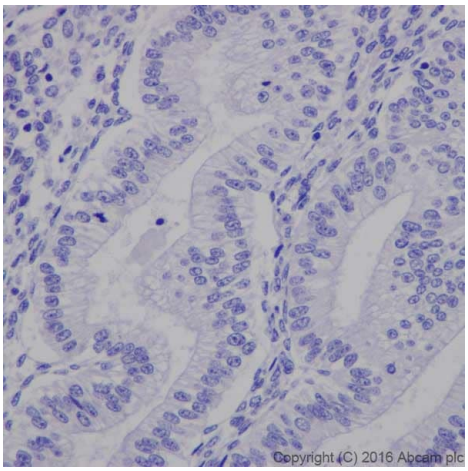
Lane 1: HEK-293 whole cell lysate transfected with CRISPR-Cas9 (*J7RUA5, Staphylococcus aureus subsp. aureus*) with Myc-His tag 10µg (Input).

Lane 2: ab203933 IP in HEK-293 whole cell lysate transfected with CRISPR-Cas9 (*J7RUA5, Staphylococcus aureus subsp. aureus*) with Myc-His tag.

Lane 3: Rabbit IgG,monoclonal [EPR25A] - Isotype Control (**ab172730**) instead of ab203933 in HEK-293 whole cell lysate transfected with CRISPR-Cas9 (*J7RUA5, Staphylococcus aureus subsp. aureus*) with Myc-His tag.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 1 second.



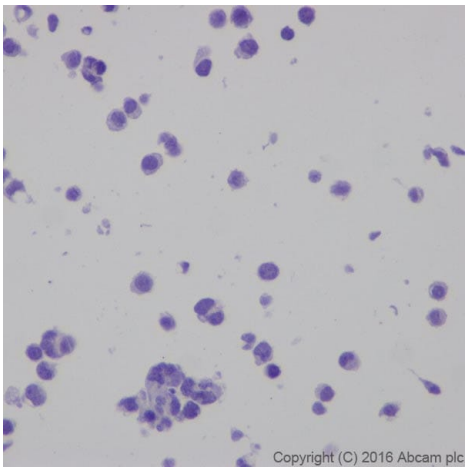
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CRISPR-Cas9 antibody [EPR19799] (ab203933)

Immunohistochemical analysis of paraffin-embedded Human endometrium tissue labeling CRISPR-Cas9 with ab203933 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

No staining on Human endometrium is observed.

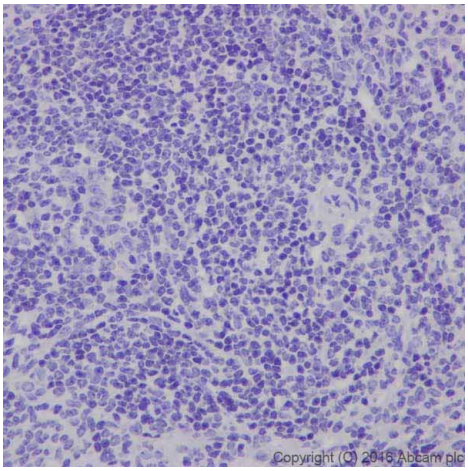
Counter stained with Hematoxylin.

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



Immunocytochemistry - Anti-CRISPR-Cas9 antibody [EPR19799] (ab203933)

Immunocytochemical analysis of agarose-embedded HeLa (Human epithelial cell line from cervix adenocarcinoma) cells transfected with blank pcDNA3.1(+)-Myc-His vector labeling CRISPR-Cas9 with ab203933 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.
No staining on HeLa cells transfected with blank pcDNA3.1(+)-Myc-His vector.
Counter stained with Hematoxylin.



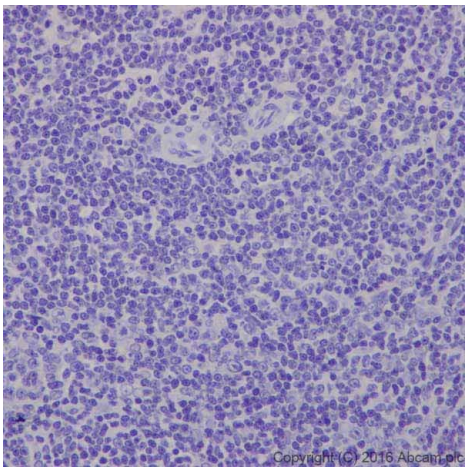
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CRISPR-Cas9 antibody [EPR19799] (ab203933)

Immunohistochemical analysis of paraffin-embedded Mouse spleen tissue labeling CRISPR-Cas9 with ab203933 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

No staining on mouse spleen is observed.

Counter stained with Hematoxylin.

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



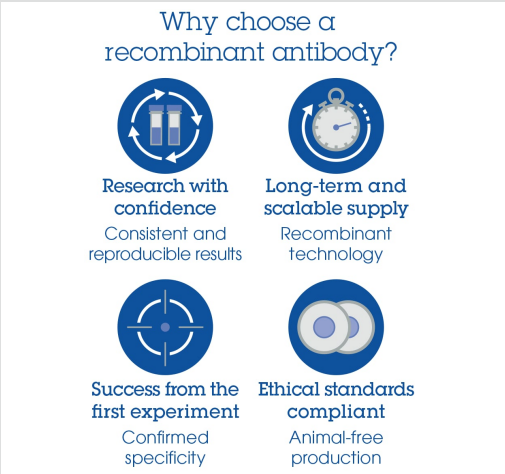
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CRISPR-Cas9 antibody [EPR19799] (ab203933)

Immunohistochemical analysis of paraffin-embedded Rat spleen tissue labeling CRISPR-Cas9 with ab203933 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

No staining on rat spleen is observed.

Counter stained with Hematoxylin.

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



Anti-CRISPR-Cas9 antibody [EPR19799] (ab203933)

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