abcam

Product datasheet

Anti-CRISPR-Cas9 antibody [7A9-3A3] ab191468

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Overview

Product name	Anti-CRISPR-Cas9 antibody [7A9-3A3]
Description	Mouse monoclonal [7A9-3A3] to CRISPR-Cas9
Host species	Mouse
Specificity	Ab191468 detects Cas9 and dCas9. This product has not been tested against SaCas9. Please contact Abcam Scientific Support for more information.
Tested applications	**Suitable for:** ICC/IF, WB
Species reactivity	**Reacts with:** Recombinant fragment **Predicted to work with:** Streptococcus pyogenes
Immunogen	Recombinant fragment corresponding to Streptococcus pyogenes CRISPR-Cas9 (N terminal).
Positive control	WB: S2 cells and NIH 3T3 whole cell lysate transfected with CRISPR-Cas9 ICC-IF: NIH/3T3-Cas9 transfected cells.
General notes	This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact **orders@abcam.com**. The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Store at -20°C long term.
Storage buffer	pH: 7.40 Preservative: 0.02% Sodium azide Constituent: PBS Some batches contain 6.97% L-Arginine as a stabilizing agent. For lot-specific buffer information, please contact our Scientific Support team.

Purity	Protein G purified
Clonality	Monoclonal
Clone number	7A9-3A3
Isotype	IgG1
Light chain type	kappa

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab191468 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/IF	★★★★☆ (3)	Use a concentration of 5 - 10 µg/ml.
WB	★★★★★ (6)	Use a concentration of 1 - 5 µg/ml. Detects a band of approximately 160 kDa (predicted molecular weight: 160 kDa). We recommend using 3% milk as the blocking agent for Western blot.

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 [7A9-3A3] (ab191468)

All lanes : Anti-CRISPR-Cas9 antibody [7A9-3A3] (ab191468) at 5 µg/ml

Lane 1 : NIH 3T3 whole cell lysate
Lane 2 : NIH 3T3 overexpressing Cas9 whole cell lysate

Lysates/proteins at 20 µg per lane.

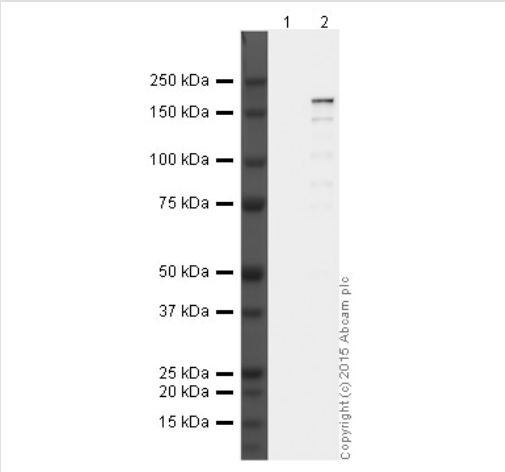
Secondary
All lanes : Goat polyclonal to Mouse IgG - H&L - Pre-Adsorbed (HRP) at 1/5000 dilution

Predicted band size: 160 kDa
Observed band size: 160 kDa

Exposure time: 4 minutes

Gel type: MOPS

Blocking buffer: 3% milk block



Western blot - Anti-CRISPR-Cas9 antibody [7A9-3A3] (ab191468)

All lanes : Anti-CRISPR-Cas9 antibody [7A9-3A3] (ab191468) at 5 µg/ml

Lane 1 : S2 non-transfected cell lysate
Lane 2 : S2 cells transfected with CRISPR-Cas9 plasmid

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat polyclonal to Mouse IgG - H&L - Pre-Adsorbed (HRP) at 1/5000 dilution

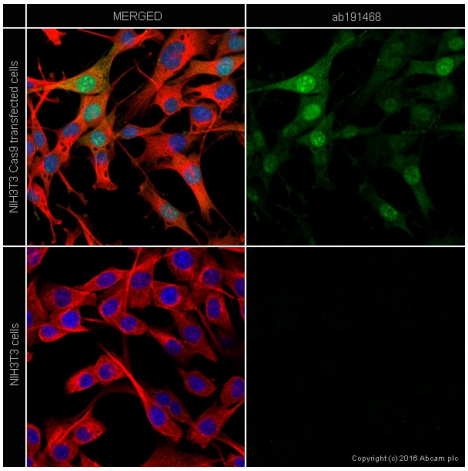
Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 160 kDa
Observed band size: 160 kDa

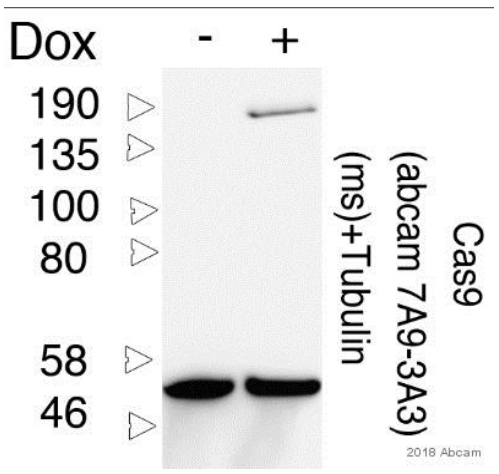
Exposure time: 4 minutes

We recommend using 3% milk as the blocking agent in Western Blot.



Immunocytochemistry/ Immunofluorescence - Anti-CRISPR-Cas9 antibody [7A9-3A3] (ab191468)

ab191468 stained in NIH3T3 cells. Untreated and Cas9 transfected cells were fixed with 4% paraformaldehyde (10min) at room temperature and incubated with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% triton for 1h at room temperature to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody ab191468 at 10µg/ml and **ab6046** (Rabbit polyclonal to beta tubulin) at 1ug/ml overnight at +4°C. The secondary antibodies were **ab150117** (colored green) used at 1 ug/ml and **ab150087** (pseudo-colored red) used at 2ug/ml for 1hour at room temperature. DAPI was used to stain the cell nuclei (colored blue) at a concentration of 1.43µM for 1hour at room temperature.



Western blot - Anti-CRISPR-Cas9 antibody [7A9-3A3] (ab191468)

This image was courtesy of an anonymous AbReview

Lane 1: HeLa inducible Cas9 cell line in the absence of Doxcycline (-DOX)

Lane 2: HeLa inducible Cas9 cell line in the treated for 24 hours in the presence of 1ug/ml of Doxcycline (+DOX).

Blot was probed with Anti-CRISPR-Cas9 antibody [7A9-3A3] (ab191468) 1:2000 and Anti-Tubulin Antibody DM1A (Sigma) as loading control. Shows specific band at 170kDA in Dox treated cells and tubulin band at 50kDa. Lysis and running Cells lysed in 1.5X Lammeli buffer +0.15M DTT syringed 10X with 25g needle and then boiled 100 degrees C for 10 minutes. 10ug of sample run out on 4-12% Bis Tris Nupage gel in 1X MOPS buffer WB protocol Western blot was performed by wet transfer of gel onto nitrocellulose membrane 100V for 2 h 4 degrees. Blocked in 5% milk PBST for 1 h room temp (1X PBS + 0.1% tween 20) incubated 24h at 4 degrees in 5% PBST with primary antibodies 1:2000 Anti-CRISPR-Cas9 antibody [7A9-3A3] (ab191468) as well as 1:3000 Anti Tubulin Mouse monoclonal DM1A antibody (Sigma) Washed 3X PBST IgG anti mouse HRP 1:5000 (Jackson immunoscience)

Washed 3x PBST Washed

HeLa cell line contains Cas9 under the control of a Doxcycline/Tetracycline inducible promotor (Tet on) Specific band at 170 kDa appears upon treatment with Doxcycline 1ug/ml for 24hours.1X PBS Developed using ECL prime kit (GE healthcare).

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