Store at -20°C

#14697 Cas9 (7A9-3A3) Mouse mAb

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Entrez-Gene ID #901176
UniProt ID #Q99ZW2

rev. 09/27/17

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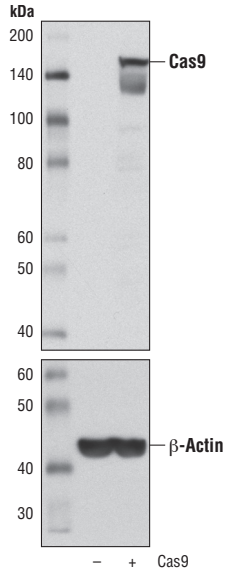
Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IHC-P, IF-IC, F Transfected	All	160 kDa	Mouse IgG1**

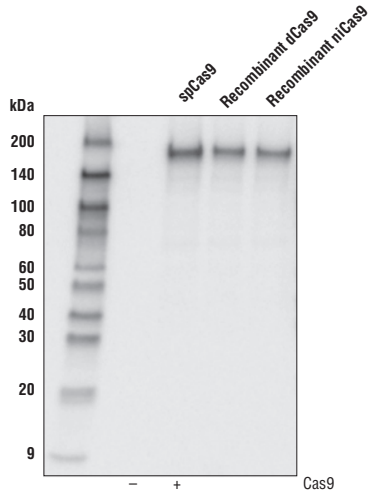
Background: The CRISPR associated protein 9 (Cas9) is an RNA-guided DNA nuclease and part of the *Streptococcus pyogenes* CRISPR antiviral immunity system that provides adaptive immunity against extra chromosomal genetic material (1). The CRISPR antiviral mechanism of action involves three steps: (i), acquisition of foreign DNA by host bacterium; (ii), synthesis and maturation of CRISPR RNA (crRNA) followed by the formation of RNA-Cas nuclease protein complexes; and (iii), target interference through recognition of foreign DNA by the complex and its cleavage by Cas nuclease activity (2). The type II CRISPR/Cas antiviral immunity system provides a powerful tool for precise genome editing and has potential for specific gene regulation and therapeutic applications (3). The Cas9 protein and a guide RNA consisting of a fusion between a crRNA and a trans-activating crRNA (tracrRNA) must be introduced or expressed in a cell. A 20-nucleotide sequence at the 5' end of the guide RNA directs Cas9 to a specific DNA target site. As a result, Cas9 can be "programmed" to cut various DNA sites both *in vitro* and in cells and organisms. CRISPR/Cas9 genome editing tools have been used in many organisms, including mouse and human cells (4,5). Research studies demonstrate that CRISPR can be used to generate mutant alleles or reporter genes in rodents and primate embryonic stem cells (6-8).

Specificity/Sensitivity: Cas9 (7A9-3A3) Mouse mAb recognizes transfected levels of total Cas9 protein.

Source/Purification: Monoclonal antibody is produced by immunizing animals with recombinant protein specific to the amino terminus of Cas9 from *Streptococcus pyogene*.



Western blot analysis of extracts from 293T cells, mock transfected (-) or transfected with a construct expressing Cas9 (+), using Cas9 (7A9-3A3) Mouse mAb (upper) and β-Actin (13E5) Rabbit mAb #4970 (lower).



Western blot analysis of extracts from 293T cells, mock transfected (-), or transfected with a construct expressing Cas9 protein isolated from S.pyogenes (spCas9) (+), or recombinant nuclease-deficient Cas9 (dCas9) protein, or recombinant Cas9 nickase (niCas9) protein, using Cas9 (7A9-3A3) Mouse mAb

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. *Do not aliquot the antibody.*

***Species cross-reactivity is determined by western blot.**

****Anti-mouse secondary antibodies must be used to detect this antibody.**

Recommended Antibody Dilutions:

Western blotting	1:1000
Immunohistochemistry (Paraffin)	1:100†
Unmasking buffer:	Citrate
Antibody diluent:	SignalStain® Antibody Diluent #8112
Detection reagent:	SignalStain® Boost (HRP) #8125

†*Optimal IHC dilutions determined using SignalStain® Boost IHC Detection Reagent.*

Immunofluorescence (IF-IC)	1:800
Flow Cytometry	1:50

For product specific protocols and a complete listing of recommended companion products please see the product web page at www.cellsignal.com

Background References:

(1) Horvath, P. and Barrangou, R. (2010) *Science* 327, 167-70.

(2) Wiedenheft, B. et al. (2012) *Nature* 482, 331-8.

(3) Singh, P. et al. (2014) *Genetics* 199, 1-15.

(4) Cong, L. et al. (2013) *Science* 339, 819-23.

(5) Mali, P. et al. (2013) *Science* 339, 823-6.

(6) Li, D. et al. (2013) *Nat Biotechnol* 31, 681-3.

(7) Shen, B. et al. (2013) *Cell Res* 23, 720-3.

(8) Niu, Y. et al. (2014) *Cell* 156, 836-43.

IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v nonfat dry milk, 1X TBS, 0.1% Tween®20 at 4°C with gentle shaking, overnight.

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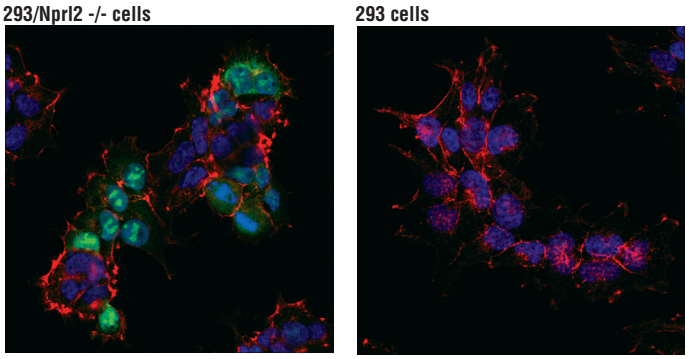
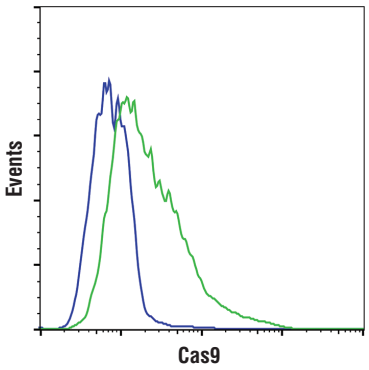
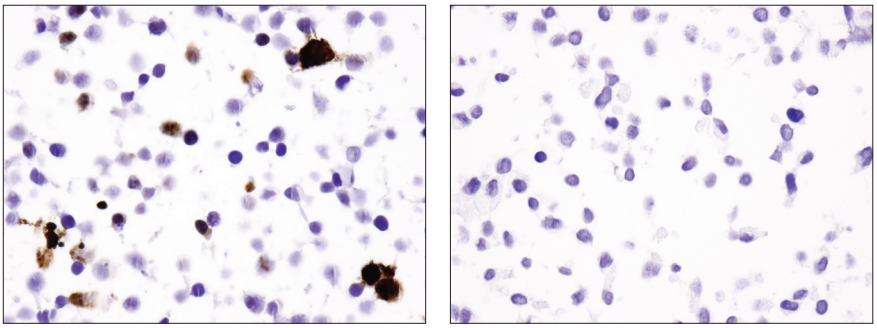
Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity:** **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



Confocal immunofluorescent analysis of Cas9 expression in Nprl2-deficient (left) and untreated 293 cells (right) using Cas9 (7A9-3A3) Mouse mAb (green). Nprl2 expression was knocked out in the Nprl2-deficient cells by transient transfection of Cas9 and Nprl2-specific guide sequences. Actin filaments were labeled with DyLight™ 554 Phalloidin #13054 (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye). 293/Nprl2 -/- cells were kindly provided by Rachel Wolfson, Lynne Chantranupong, and David Sabatini of MIT, Cambridge, MA.



Flow cytometric analysis of HEK-293 cells untreated (blue) or transfected with NPRL2 knock out (-/-) plasmid (green) using Cas9 (7A9-3A3) Mouse mAb. Anti-mouse IgG (H+L), F(ab')$_2$ fragment (Alexa Fluor® 488 Conjugate) #4408 was used as a secondary antibody.



Immunohistochemical analysis of Cas9 expression in Nprl2-deficient 293 (left, positive) and untreated 293 (right, negative) cell pellets using Cas9 (7A9-3A3) Mouse mAb. Nprl2 expression was knocked out in the Nprl2-deficient cells by transient transfection of Cas9 and Nprl2-specific guide sequences. 293/Nprl2 -/- cells were kindly provided by Rachel Wolfson, Lynne Chantranupong, and David Sabatini of MIT, Cambridge, MA.

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