Revision 4

Store at -20C

#65832

Cas9 (*S. pyogenes*) (D8Y4K) Rabbit mAb

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not for Use in Diagnostic Procedures.

Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
W, W-S, IF-F, IF-IC, FC-FP	All	Transfected Only	150	Rabbit IgG	#Q99ZW2	901176

Product Usage Information

Application	Dilution
Western Blotting	1:1000
Simple Western™	1:50 - 1:250
Immunofluorescence (Frozen)	1:50 - 1:200
Immunofluorescence (Immunocytochemistry)	1:200
Flow Cytometry (Fixed/Permeabilized)	1:50

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.

Specificity/Sensitivity

Cas9 (*S. pyogenes*) (D8Y4K) Rabbit mAb recognizes transfected levels of total Cas9 (*S. pyogenes*) protein. This antibody does not cross-react with Cas9 (*S. aureus*), FnCpf1, and AsCpf1 proteins.

Source / Purification

Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Val16 of Cas9 (*S. pyogenes*) protein.

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 extrachromosomal 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).

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. (2015) *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.

Species Reactivity

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

Western Blot Buffer

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

Applications Key

W: Western Blotting **W-S:** Simple Western™ **IF-F:** Immunofluorescence (Frozen) **IF-IC:** Immunofluorescence (Immunocytochemistry) **FC-FP:** Flow Cytometry (Fixed/Permeabilized)

Cross-Reactivity Key

All: All Species Expected

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