Revision 1

#30004 Store at +4C

Cas9 *(S. aureus)* (6H4) Mouse mAb (Alexa Fluor® 647 Conjugate)

Cell Signaling TECHNOLOGY®

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Applications:	Reactivity:	Sensitivity:	Source/Isotype:	UniProt ID:
IF-IC	All	Transfected Only	Mouse IgG2b	#J7RUA5

Product Usage Information

Application	Dilution
Immunofluorescence (Immunocytochemistry)	1:50

Storage

Supplied in PBS (pH 7.2), less than 0.1% sodium azide and 2 mg/ml BSA. Store at 4°C. *Do not aliquot the antibody. Protect from light. Do not freeze.*

Specificity/Sensitivity

Cas9 (*S. aureus*) (6H4) Mouse mAb (Alexa Fluor® 647 Conjugate) recognizes endogenous levels of total Cas9 (*S. aureus)* protein. This antibody does not cross-react with Cas9 (*S. pyogenes),* AsCpf1 (Strain *BV3L6*), and FnCpf1 (Strain *U112*) proteins.

Source / Purification

Monoclonal antibody is produced by immunizing animals with recombinant protein specific to the amino terminus of Cas9 (*S. aureus)* protein.

Description

This Cell Signaling Technology antibody is conjugated to Alexa Fluor® 647 fluorescent dye and tested in-house for direct immunofluorescent analysis in human cells. This antibody is expected to exhibit the same species cross-reactivity as the unconjugated Cas9 (*S. aureus*) (6H4) Mouse mAb #48989.

Background

The CRISPR associated protein 9 (Cas9) is an RNA-guided DNA nuclease and part of the 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).

Cas9 (*S. aureus*) is a Cas9 ortholog that is smaller, but as efficient, as the more commonly used Cas9 ortholog, Cas9 (*S. pyogenes*) (9).

Background References

1. Horvath, P. and Barrangou, R. (2010) *Science* 327, 167-70.
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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.
9. Ran, F.A. et al. (2015) *Nature* 520, 186-91.

Species Reactivity

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

Applications Key

IF-IC: Immunofluorescence (Immunocytochemistry)

Cross-Reactivity Key

All: All Species Expected

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