

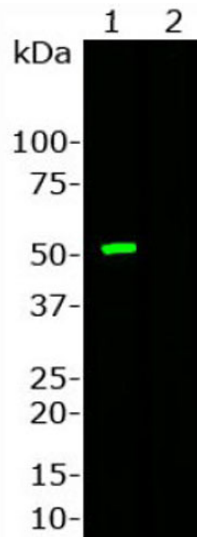
Ordering Information
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HGNC name: NA, no human homolog
RRID: AB_2572247
Immunogen: C-terminal region of *S. aureus*, amino acids 803-1053 of sequence CCK74173, expressed in and purified from *E. coli*.
Format: Purified antibody at 1mg/mL in 50% PBS, 50% glycerol plus 5mM Na₂S₂O₃
Storage: Store at 4°C for short term, for longer term at -20°C
Recommended dilutions:
 WB: 1:1,000, IF/ICC and IHC: 1:1,000-1:5,000.

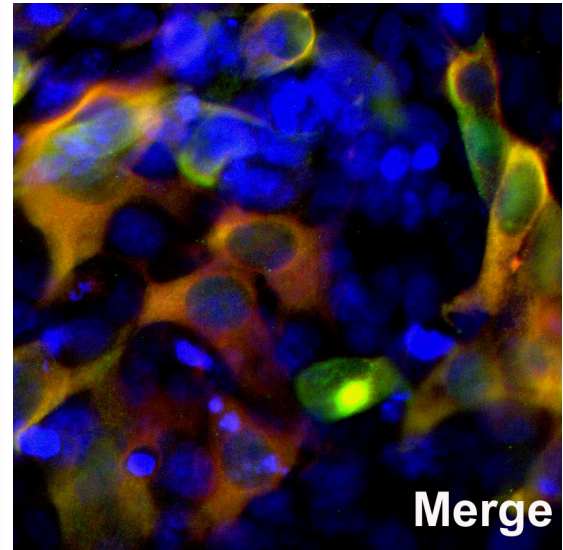
References:

1. Hsu PD, Lander ES, Zhang F. Development and Applications of CRISPR-Cas9 for Genome Engineering. *Cell* 157:1262-78 (2014).
2. Doudna J, Charpentier E. The new frontier of genome engineering with CRISPR-Cas9. *Science* 346:1077-86 (2014)
3. Long C, et al. Postnatal genome editing partially restores dystrophin expression in a mouse model of muscular dystrophy. *Science* 351:400-3 (2015).
4. Nelson CE, et al. In vivo genome editing improves muscle function in a mouse model of Duchenne muscular dystrophy. *Science* 351:403-7 (2015).
5. Tabebordbar M, et al. In vivo gene editing in dystrophic mouse muscle and muscle stem cells. *Science* 351:407-11 (2015).
6. Amoasii L, et al. Gene editing restores dystrophin expression in a canine model of Duchenne muscular dystrophy. *Science* doi:10.1126/science.aau1549 (2018).
7. Ran FA, et al. In vivo genome editing using Staphylococcus aureus Cas9. *Nature* 520:186-91 (2015).
8. Knott GJ, Doudna J. CRISPR-Cas guides the future of genetic engineering. *Science* 361:866-9 (2018).

Applications	Host	Isotype	Molecular Wt.	Species Cross-Reactivity
WB, IF/ICC, IHC	Mouse	IgG1	124kDa	Sa



Western blot analysis of HEK293 cell lysates using mouse mAb to *S. aureus* Cas9, MCA-6F7, dilution 1:1,000 in green: [1] protein standard, [2] non-transfected cells, and [3] transfected cells with a fusion construct of GFP with the amino acids 803-1053, the C-terminal of *S. aureus* Cas9. The strong band at about 53kDa corresponds to the GFP-Cas9 fusion protein expressed only in transfected cells.



HEK293 cells were transfected with a fusion construct of green fluorescent protein (GFP) with the C-terminal of *S. aureus* Cas9, amino acids 803-1053. Cells were stained with MCA-6F7 in red and GFP gives a green signal. The two signals overlap as expected. The nuclei of both transfected and untransfected cells are revealed by the DAPI DNA stain.

Background:

A recent revolution in biology has been stimulated by the discovery of CRISPR, or "Clustered Regularly Interspaced Short Palindromic Repeats" and the understanding of the "CRISPR Associated" enzymes (CAS 1,2). The CRISPR repeated sequences are found in bacterial genomes and function as part of unique bacterial immune system which contain short DNA sequences derived from viruses which have infected the bacteria. These virally derived sequences can make short RNA sequences which can hybridize with specific viral DNA and target a nuclease, such as CAS9, to the viral sequence. So CAS9 is directed to cleave the specific viral sequence and so inactivate the virus. The RNA sequence can be designed to specifically cut DNA virtually anywhere, including in the genomes of living human and other mammalian cells, allowing inexpensive gene editing with unprecedented ease. For example three groups of researchers essentially cured the disease state in a mouse model of Duchenne muscular dystrophy (3-5). A similar approach essentially cured dogs affected with a related disease state (6). Several varieties of CAS9 have been studied and there are several other related enzymes with similar properties. Much of the early work was performed with CAS9 from *Streptococcus pyogenes* which is rather large at ~158kDa, so the corresponding DNA is also rather large at about 4.2kb. This is problematic with some expression systems especially since DNA encoding RNA sequences and possibly other regulatory elements are usually required. The CAS9 gene of *Staphylococcus aureus* is significantly smaller, 3kb, producing a protein of 124kDa (7). For an excellent recent review of the various CAS family enzymes and their utility see reference 8.

The MCA-6F7 antibody was raised against the C-terminal 250 amino acids of *S. aureus* CAS9 in the sequence CCK74173. It can be used to verify expression of *S. aureus* CAS9 in cells and tissues. The antibody does not bind *S. pyogenes* CAS9. We used the same immunogen to generate rabbit and chicken polyclonals to *S. aureus*, [RPCA-CAS9-Sa](#) and [CPCA-CAS9-Sa](#).

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Abbreviation Key:

mAb—Monoclonal Antibody pAb—Polyclonal Antibody WB—Western Blot IF—Immunofluorescence ICC—Immunocytochemistry
 IHC—Immunohistochemistry E—ELISA Hu—Human Mo—Monkey Do—Dog Rt—Rat Ms—Mouse Co—Cow Pi—Pig Ho—Horse Ch—Chicken
 Dr—D. rerio Dm—D. melanogaster Sm—S. mutans Ce—C. elegans Sc—S. cerevisiae Sa—S. aureus Ec—E. coli.