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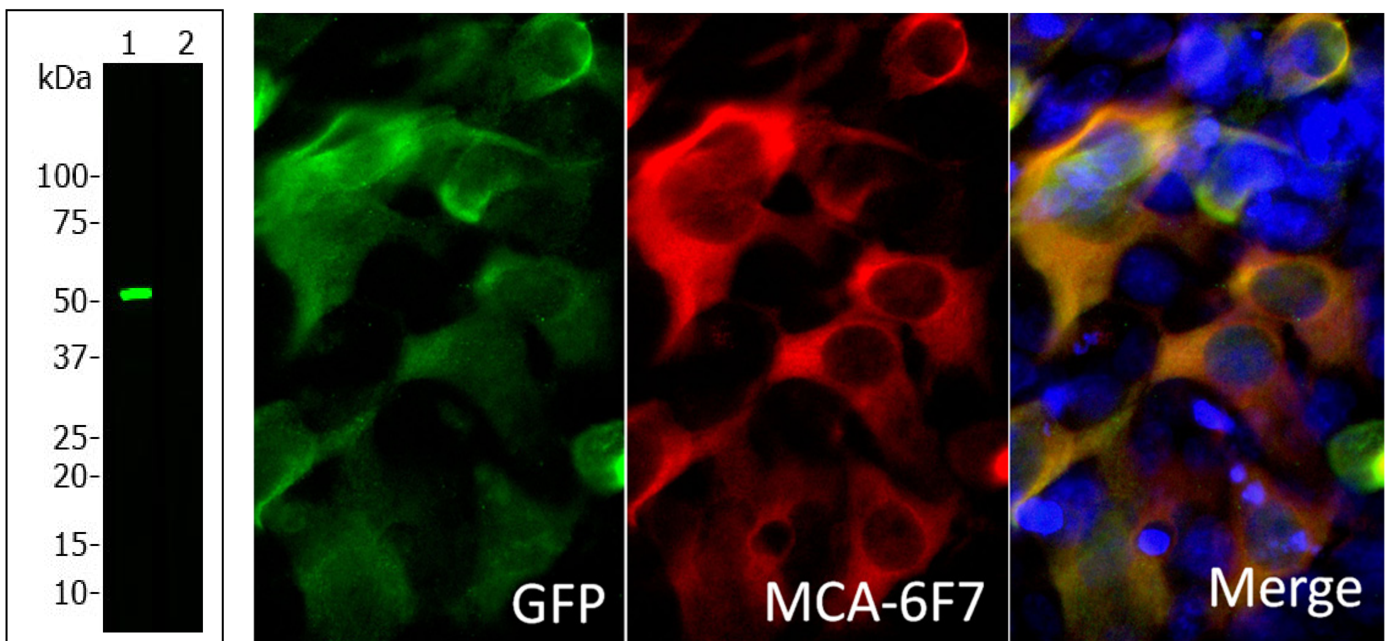
Catalogue# MCA-6F7: Mouse Monoclonal Antibody to Cas9 from *Staphylococcus aureus*

The Immunogen: A recent revolution is biology has been stimulated by the discovery of CRISPR, or "Clustered Regularly Interspaced Short Palindromic Repeats" and the understanding of their significance. These repeated sequences are found in bacterial genomes and function as part of unique bacterial immune system. Interspaced between these repeated DNA sequences are 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, if the bacteria is infected by this virus again, Cas9 can be directed to cleave the specific viral sequenceIa and so inactivate the virus. By careful design of the RNA sequence the system can be used to specifically cut DNA virtually anywhere, including in living human and other mammalian cells. This allows inexpensive gene editing with unprecedented ease, and much effort is going into refining the Cas9 for use in mammalian systems. Recent papers in this exploding field showed that it is feasible to correct genetic defects in a variety of experimental situations. For example three groups of researchers essentially cured the disease state in a mouse model of Duchenne muscular dystrophy, a disease in which point mutations or frame shifts result in the production of a truncated and non-functional form of very large muscle protein dystrophin (1). This was performed using AAV vectors on adult animals, using RNA sequences which directed cleavage of the DNA at two sites flanking the genetic defect. The normal DNA repair mechanisms in some cases annealed the two cut sites leaving out the defective region. This allowed the production of a slightly shorter but still functional dystrophin protein. Several varieties of Cas9 have been studied and there appear to be several other related enzymes with similar properties in bacteria. Much of the early work was performed with Cas9 from *Streptococcus pyogenes*. The *S. pyogenes* protein is rather large at 1,368 amino acids, ~158kDa, so the corresponding DNA is also rather large at about 4.2 kb. This will not fit easily into some expression systems especially since DNA encoding RNA sequences and possibly other regulatory elements are usually required. In one recent study, Ran et al. (2), a group in the Broad Institute, searched for the smallest possible Cas9 across known bacterial genomes and found that the version expressed in *Staphylococcus aureus* was significantly smaller, at about 3 kb, producing a protein of 124kDa (2). Our antibody is a monoclonal raised against the C-terminal 251 amino acids of of the *Staphylococcus aureus* protein and binds this protein transfected into cells on western blots and in immunocytochemistry. The homologous region of the *Streptococcus pyogenes* is not closely related in amino acid sequence and, as expected, this antibody does not recognize that protein.



Left: Western blot analysis of MCA-6F7. Lane 1: HEK293 cells which overexpressed fusion protein containing GFP and c-terminus of Cas9 from *S. aureus*. Lane 2: Non-transfected HEK293 cells. There is a strong clean band at about 53 kDa corresponding to GFP-Cas9 fusion protein, which is absent in non-transfected cells. MCA-6F7 antibody is diluted

at 1: 1,000. **Right:** Transfected HEK293 cells with GFP-Cas9-SA fusion protein were stained with MCA-6F7 antibody. GFP is expressed in the transfected cells (green). GFP-Cas9 fusion protein is stained with MCA-6F7 antibody at 1:1,000 dilution (red). Only transfected cells are reactive with MCA-6F7 antibody, which appear in a yellow-orange color (merge). Nuclei are visualized in blue with Hoechst staining.

Antibody Characteristics: MCA-6F7 is a mouse IgG1 class antibody. The antibody was raised against the C-terminal region of Cas9 from *Staphylococcus aureus*. The AA sequences corresponding to 803-1053 (GenBank: CCK74173.1) of the protein was expressed in and purified from *E. Coli*. The antibody solution is purified from tissue culture supernatant at a concentration of 1 mg/mL in 50% glycerol.

Suggestions for use: The antibody solution can be used at dilutions of 1:1,000 in Western blot, and 1:1,000-1:5,000 for ICC/IF and IHC.

Storage Instructions: Shipped on ice. Please store at 4°C for regular uses. For long term storage, please leave frozen at -20°C and avoid freeze/thaw cycles.

Limitations: This product is for research use only and is not approved for use in humans or in clinical diagnosis.

References:

1. Nelson CE, Hakim CH, Ousterout DG, Thakore PI, Moreb EA, Castellanos Rivera RM, Madhavan S, Pan X, Ran FA, Yan WX, Asokan A, Zhang F, Duan D, Gersbach CA. In vivo genome editing improves muscle function in a mouse model of Duchenne muscular dystrophy. Science. 2016 Jan 22;351(6271):403-7.

2. Ran FA, Cong L, Yan WX, Scott DA, Gootenberg JS, Kriz AJ, Zetsche B, Shalem O, Wu X, Makarova KS, Koonin EV, Sharp PA, Zhang F. *In vivo* genome editing using *Staphylococcus aureus* Cas9. Nature 520:186-91 (2015).

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