

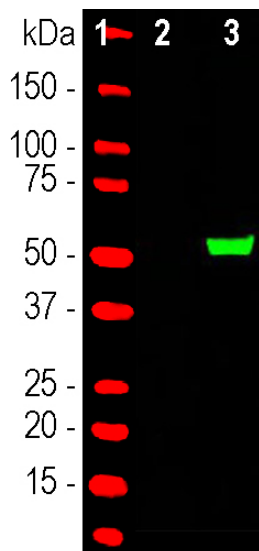
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**HGNC Name:** N.A.  
**UniProt:** J7RUA5  
**RRID:** AB\_2744685  
**Immunogen:** CAS9 from *S. aureus* expressed in and purified from *E. coli*  
**Format:** Antibody is supplied as rabbit serum  
**Storage:** Storage for short term at 4°C recommended, for longer term at -20°C, minimize freeze/thaw cycles  
**Recommended dilutions:**  
WB: 1:1,000-1:5,000. IF/ICC and IHC: 1:5,000

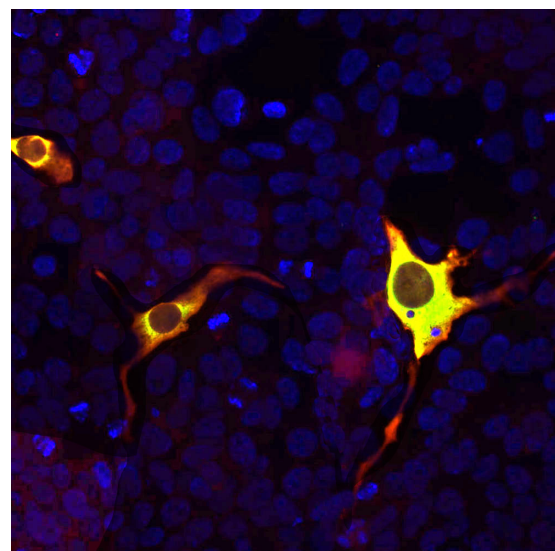
### References:

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2. Doudna JA, 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).
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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	Rabbit		124kDa	Sa



Western blot analysis of HEK293 cell lysates using rabbit pAb to *S. aureus* CAS9, RPCA-CAS9-Sa: [1] protein standard (red), [2] nontransfected cells and [3] transfected cells with GFP-CAS9 (C-terminal 803-1053 amino acids of *S. aureus* CAS9) fusion construct. The band at about 53kDa corresponds to the GFP-CAS9 fusion protein.



HEK293 cells were transfected with a construct including the C-terminal 250 amino acids of *S. aureus* CAS9 fused to GFP and stained with RPCA-CAS9-Sa in red. Transfected cells express the green fusion protein and bind the antibody in red, producing a yellow signal. Nuclear DNA in transfected and non-transfected cells is revealed with the blue DNA stain DAPI.

### 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 (6). For an excellent recent review of the various CAS family enzymes and their utility see reference 8.

The RPCA-CAS9-Sa 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 CAS9 in cells and tissues. The antibody does not bind *S. pyogenes* CAS9. We used the same *S. aureus* immunogen to generate chicken polyclonal and a mouse monoclonal antibodies to *S. pyogenes* CAS9 [CPCA-CAS9-Sp](#) and [MCA-6F7](#).

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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*.