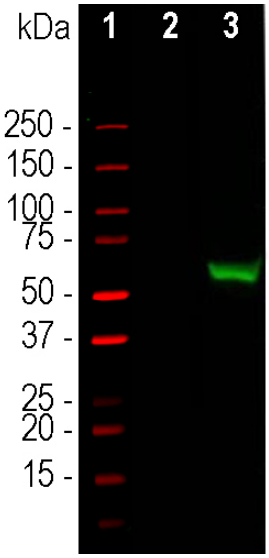
CAS9 from *S. aureus* Chicken Polyclonal Antibody

CPCA-CAS9-Sa

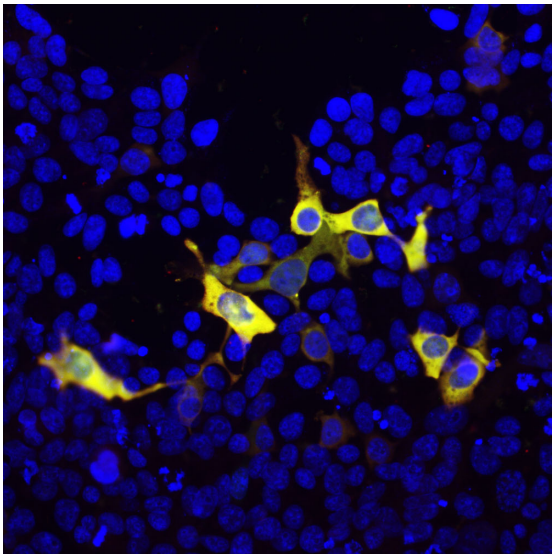
Ordering Information
Web *www.encorbio.com*
Email *admin@encorbio.com*
Phone *352-372-7022*
Fax *352-372-7066*

HGNC Name: *NA*
UniProt: *J7RUA5*
RRID: *AB_2572245*
Immunogen: *C-terminal region of S. aureus CAS9 expressed in and purified from E. coli.*
Format: *Concentrated IgY preparation in PBS plus 0.02% NaN_3*
Storage: *Stable at 4°C for at least one year. Mix 1:1 with 100% glycerol and store at -20°C for longer term storage*
Recommended dilutions:
WB: 1:1,000. IF/ICC and IHC:1: 1,000-1:5,000

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



Western blot analysis of HEK293 cell lysates using chicken pAb to S. aureus CAS9, CPCA-CAS9-Sa: [1] protein standard (red), [2] non-transfected 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 N-terminal 608 amino acids of *S. aureus* CAS9 fused to GFP and stained with CPCA-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 CPCA-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 *S. aureus* CAS9 in cells and tissues. The antibody does not bind *S. pyogenes* CAS9 due to the low level of sequence homology. We used the same *S. aureus* immunogen to generate a rabbit polyclonal and a mouse monoclonal to *S. aureus*, RPCA-CAS9-Sa and MCA-6F7. EnCor also generated a mouse monoclonal and a rabbit polyclonal to the larger CAS9 molecule of *Streptococcus pyogenes*, RPCA-CAS9-Sp and MCA-3F9.

References:

1. Hsu PD, Lander ES, Zhang F. Development and Applications of CRISPR-Cas9 for Genome Engineering. Cell 157:1262-78 (2014). 2. Doudna1 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). 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).

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.