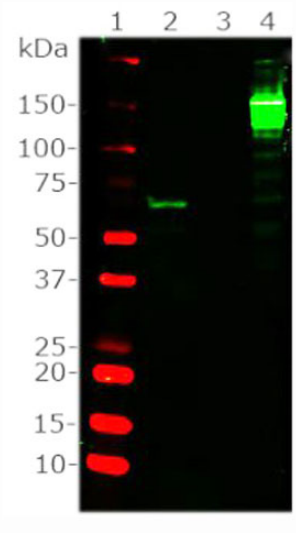
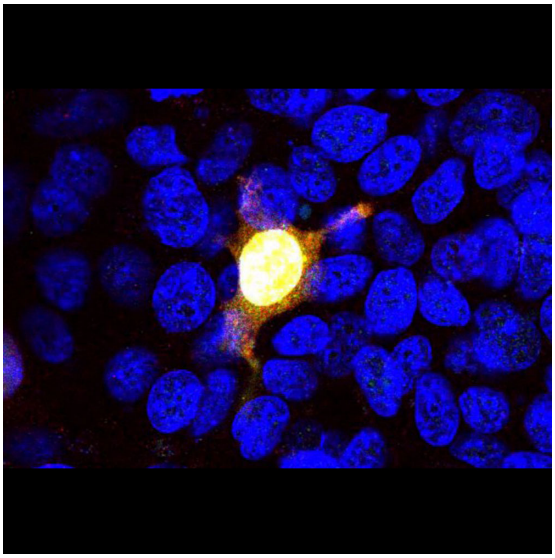
CAS9 from *S. pyogenes* Mouse Monoclonal Antibody

MCA-3F9

Applications	Host	Isotype	Molecular Wt.	Species Cross-Reactivity
WB, IF/ICC, IHC	Mouse	IgG	160kDa	Sp



Western blot analysis of MCA-3F9. [1] Protein size marker with size in kiloDaltons, [2] blot of crude lysate from HEK293 cells transfected with the MCA-3F9 immunogen, the N-terminal 1-608 amino acids of Cas9, [3] non-transfected control HEK293 cell extract, [4] 40ng full length S. pyogenes Cas9. Blots were probed with MCA-3F9 at 1:1,000 dilution and as expected, the antibody recognizes the immunogen and full length Cas9 *S. pyogenes* protein at 160kDa.



HEK293 cells were transfected with a construct including the N-terminal 608 amino acids of *S. pyogenes* CAS9 fused to GFP and stained with MCA-3F9 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 (7). For an excellent recent review of the various CAS family enzymes and their utility see reference 8.

The MCA-3F9 antibody was made against the N-terminal region of *S. pyogenes* CAS9. It can be used to verify the expression of *S. pyogenes* CAS9 on blots, in cells and tissues. The same immunogen was used to generate a rabbit polyclonal antibody to CAS9, RPCA-CAS9-Sp. EnCor also manufactures antibodies against the smaller CAS9 homologue from *S. aureus*.

Ordering Information
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HGNC name: *NA, no human homolog*
RRID: *AB_2572246*
Immunogen: *N-terminal region, amino acids 1-608 of Cas9 sequence CDJ55032.1 from Streptococcus pyogenes, expressed in and purified from E. coli.*
Format: *Purified antibody at 1mg/mL in 50% PBS, 50% glycerol plus 5mM NaN_3*
Storage: *Store at 4°C for short term, for longer term at -20°C.*
Recommended dilutions:
WB: 1:1,000-1: 2,000 on CAS9 transfected cells and 1:10,000-20,000 on pure full length CAS9 protein.
IF/ICC:1:1,000-2,000

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.