CasΦ/Cas 12j Mouse Monoclonal Antibody

MCA-5F95

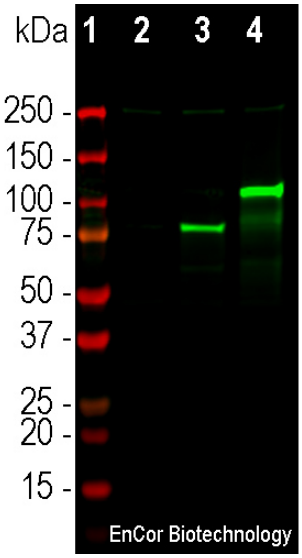
Ordering Information
Web *www.encorbio.com*
Email *admin@encorbio.com*
Phone *352-372-7022*
Fax *352-372-7066*

HGNC Name: *NA*
UniProt: *NA*
RRID: *AB_2889159*
Immunogen: *Recombinant CasΦ/Cas-12j expressed in and purified from E. coli*
Format: *Purified antibody at 1mg/mL in 50% PBS, 50% glycerol plus 5mM NaN_3*
Storage: *Shipped on ice. Store at 4°C for short term, for longer term at -20°C. Avoid freeze / thaw cycles.*
Recommended dilutions:
WB: 1:1,000-1:2,000. ICC/IF: 1:1,000

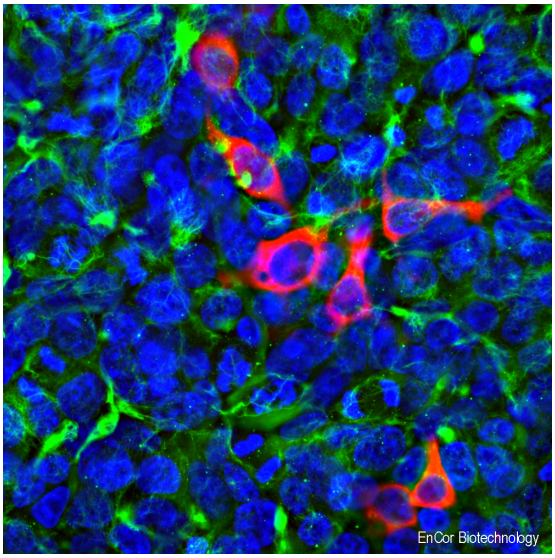
References:

1. Al-Shayeb, B et al. Clades of huge phages from across Earth's ecosystems. Nature DOI: 10.1038/s41586-020-2007-4 578:425-531 (2020).
2. Pausch, P. CRISPR-CasΦ from huge phages is a hypercompact genome editor. Science DOI: 10.1126/science.abb1400 369:333-337 (2020).

Applications	Host	Isotype	Molecular Wt.	Species Cross-Reactivity
WB, ICC/IF	Mouse	IgG1	90kDa	NA



Western blot analysis of HEK293 cell lysates using mouse mAb to CasΦ protein, MCA-5F95, dilution 1:1,000 in green: [1] protein molecular weight standard, [2] non-transfected cells, [3] cells transfected with pCI-Neo-Mod vector containing full length CasΦ cDNA, and [4] cells transfected with pCI-Neo-GFP vector containing full length CasΦ cDNA. The band at about 90kDa demonstrates expression of CasΦ protein, and the band at about 120kDa corresponds to GFP-CasΦ fusion protein.



Immunofluorescent analysis of HEK293 cells transfected with pCI-Neo-Mod vector (5) including DNA encoding the CasΦ protein, stained with mouse mAb against CasΦ, MCA-5F95, dilution 1:2,000, in red, and costained with rabbit pAb to vimentin, RPCA-Vim, dilution 1:2,000, in green. The blue is Hoechst staining of nuclear DNA. The MCA-5F95 antibody reveals expression of the CasΦ protein only in transfected cells. The vimentin antibody stains the cytoskeletal intermediate filament network in all cells.

Background:

There has been much recent interest in gene editing and other genetic manipulations by CRISPR-Cas family enzymes. Recent ecosystem and microbiome DNA sequencing and assembly characterized genomes of "huge phages" or megaphages, see Al-Shayeb et al. Nature 578:425-431 (2020) and showed that they contained novel Cas family enzymes. The lab of Nobel prize winner Jennifer Doudna characterized these Cas enzymes and showed that they could be used to perform CRISPR, but had the significant advantage that they were much smaller in molecular size than other Cas family enzymes such as the widely used Cas9 enzymes from *S. pyogenes* and *S. aureus*, see Pausch, P. et al. Science 369:333-337 (2020). One of these enzymes is referred to as CasΦ and also known as Cas-12j and was the focus of the Pausch et al. paper. The use of such smaller Cas family enzymes leaves more room for other nucleic acid sequences in AAV and other viral vectors which typically have a limited DNA capacity, thus allowing more versatility for new CRISPR based manipulations.

The MCA-5F95 antibody was made against our recombinant construct of the full sequence of CasΦ-2 from Pausch et al. 2020. The antibody works well on western blots of crude homogenates of HEK293 cells transfected with the Cas-Φ-2 DNA, cleanly producing the appropriate sized band. In addition such transfected cells show clean and strong cytoplasmic staining by immunofluorescence staining with this antibody. We also supply a rabbit polyclonal antibody to this protein, RPCA-Cas12j.

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.