

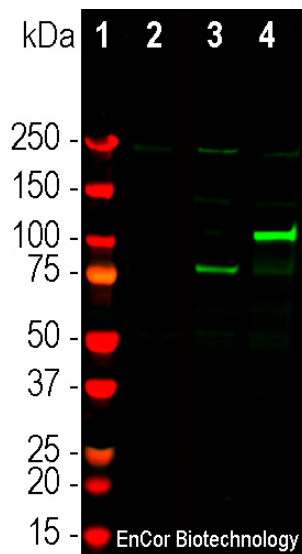
Ordering Information
 Web www.encorbio.com
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HGNC Name: NA
UniProt: NA
RRID: 2889160
Immunogen: Full length Cas Φ -2 expressed in and purified from *E. coli*.
Format: Purified antibody at 1mg/mL in 50% PBS, 50% glycerol plus 5mM NaN₃
Storage: Stable at 4°C for one year, for longer term store at -20°C
Recommended dilutions:
 WB: 1:1,000-2,000. IF/ICC 1:2,000-5,000

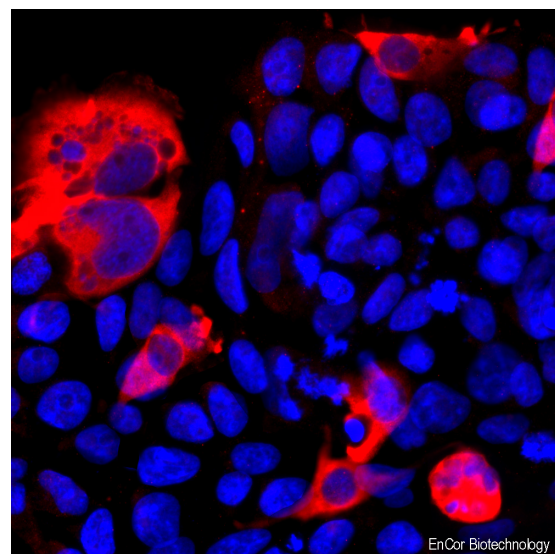
References:

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-Cas Φ 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, IF/ICC	Rabbit	IgG1	~90kDa	NA



Western blot analysis of HEK293 cell lysates using rabbit pAb to Cas Φ protein, RPCA-Cas12j, dilution 1:1,000 in green: [1] protein standard, [2] non-transfected cells, [3] cells transfected with pCI-Neo-Mod vector containing full length Cas Φ cDNA, and [4] cells transfected with pCI-Neo-GFP vector containing full length Cas Φ cDNA. The band at about 90kDa demonstrates expression of Cas Φ protein, and the band at about 120kDa corresponds to the expected GFP-Cas Φ fusion protein.



Immunofluorescent analysis of HEK293 cells transfected with pCI-Neo-Mod vector including DNA encoding full length Cas Φ protein and stained with rabbit pAb against Cas Φ , RPCA-Cas12j, in red. The blue is Hoechst staining of nuclear DNA. The antibody reveals cytoplasmic expression of the Cas Φ protein only in transfected 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 Cas- Φ and also known as Cas-12j and was the focus of the Pausch et al. paper. The use of such smaller 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 RPCA-Cas12j antibody was made against our recombinant construct of the full sequence of Cas Φ -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 mouse monoclonal antibody to this protein, MCA-5F95.

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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*.