Cas9 Nuclease, *S. pyogenes*

1-800-632-7799
info@neb.com
www.neb.com

M0386S 002150717071

M0386S

RR 37°

50 pmol	**1,000 nM**	**Lot: 0021507**
RECOMBINANT	**Store at –20°C**	**Exp: 7/17**

Description: Cas9 Nuclease, *S. pyogenes*, is an RNA-guided endonuclease that catalyzes site-specific cleavage of double stranded DNA. The location of the break is within the target sequence 3 bases from the NGG PAM (Protospacer Adjacent Motif) (1). The PAM sequence, NGG, must follow the targeted region on the opposite strand of the DNA with respect to the region complementary sgRNA sequence.

Source: An *E. coli* strain that carries the cloned Cas9 gene from *Streptococcus pyogenes*

Supplied in: 300 mM NaCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT and 50% glycerol. (pH 7.4 @ 25°C).

Reagents Supplied with Enzyme:
10X Cas9 Nuclease Reaction Buffer

Reaction Conditions: 1X Cas9 Nuclease Reaction Buffer. Incubate at 37°C.

1X Cas9 Nuclease Reaction Buffer:
20 mM HEPES
100 mM NaCl
5 mM $MgCl_2$
0.1 mM EDTA
pH 6.5 @ 25°C

Diluent Compatibility: Diluent Buffer B
300 mM NaCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, 500 µg/ml BSA and 50% glycerol. (pH 7.4 @ 25°C).

Quality Control Assays

Protein Purity (SDS-PAGE): Cas9 Nuclease, *S. pyogenes* is > 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

RNase Activity (Extended Digestion): A 10 µl reaction in Cas9 Nuclease Reaction Buffer containing 40 ng of labeled RNA and 1 picomole of Cas9 Nuclease, *S. pyogenes* is incubated at 37°C. After incubation for 4 hours, > 90% of the substrate RNA remains intact as determined by agarose gel electrophoresis.

Endonuclease Activity (Nicking): A 50 µl reaction in Cas9 Nuclease Reaction Buffer containing 1 picomole of Cas9 Nuclease, *S. pyogenes* with 1 µg ϕX174 RF I DNA for 4 hours at 37°C resulted in < 10% conversion to RF II as determined by agarose gel electrophoresis.

Exonuclease Activity (Radioactivity Release): A 50 µl reaction in Cas9 Nuclease Reaction Buffer containing 1 µg of a mixture of single and double-stranded [^{3}H] *E. coli* DNA and 1 picomole of Cas9 Nuclease, *S. pyogenes* incubated for 4 hours at 37°C releases < 0.1% of the total radioactivity.

Non-Specific DNase Activity (16 hour): A 50 µl reaction in Cas9 Nuclease Reaction Buffer containing 1 µg of λ DNA and 1 picomole of Cas9 Nuclease, *S. pyogenes* incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

Functional Test (Cas9 Nuclease, *S. pyogenes* Targeted Digestion): A 30 µl reaction in 1X Cas9 Nuclease Reaction Buffer containing 1 nM PvuII linearized pBR322 DNA (one targeted site CGCTTGTTTCGGCGTGGGTA), 20 nM SgRNA and 20 nM Cas9 Nuclease, *S. pyogenes* incubated for 1 hour at 37°C results in 95% digestion of the substrate DNA as determined by agarose gel electrophoresis.

Note: 1,000 nM is equal to 159 ng/µl.

(see other side)

CERTIFICATE OF ANALYSIS

Cas9 Nuclease, *S. pyogenes*

1-800-632-7799
info@neb.com
www.neb.com

M0386S 002150717071

M0386S

RR 37°

50 pmol	**1,000 nM**	**Lot: 0021507**
RECOMBINANT	**Store at –20°C**	**Exp: 7/17**

Description: Cas9 Nuclease, *S. pyogenes*, is an RNA-guided endonuclease that catalyzes site-specific cleavage of double stranded DNA. The location of the break is within the target sequence 3 bases from the NGG PAM (Protospacer Adjacent Motif) (1). The PAM sequence, NGG, must follow the targeted region on the opposite strand of the DNA with respect to the region complementary sgRNA sequence.

Source: An *E. coli* strain that carries the cloned Cas9 gene from *Streptococcus pyogenes*

Supplied in: 300 mM NaCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT and 50% glycerol. (pH 7.4 @ 25°C).

Reagents Supplied with Enzyme:
10X Cas9 Nuclease Reaction Buffer

Reaction Conditions: 1X Cas9 Nuclease Reaction Buffer. Incubate at 37°C.

1X Cas9 Nuclease Reaction Buffer:
20 mM HEPES
100 mM NaCl
5 mM $MgCl_2$
0.1 mM EDTA
pH 6.5 @ 25°C

Diluent Compatibility: Diluent Buffer B
300 mM NaCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, 500 µg/ml BSA and 50% glycerol. (pH 7.4 @ 25°C).

Quality Control Assays

Protein Purity (SDS-PAGE): Cas9 Nuclease, *S. pyogenes* is > 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

RNase Activity (Extended Digestion): A 10 µl reaction in Cas9 Nuclease Reaction Buffer containing 40 ng of labeled RNA and 1 picomole of Cas9 Nuclease, *S. pyogenes* is incubated at 37°C. After incubation for 4 hours, > 90% of the substrate RNA remains intact as determined by agarose gel electrophoresis.

Endonuclease Activity (Nicking): A 50 µl reaction in Cas9 Nuclease Reaction Buffer containing 1 picomole of Cas9 Nuclease, *S. pyogenes* with 1 µg ϕX174 RF I DNA for 4 hours at 37°C resulted in < 10% conversion to RF II as determined by agarose gel electrophoresis.

Exonuclease Activity (Radioactivity Release): A 50 µl reaction in Cas9 Nuclease Reaction Buffer containing 1 µg of a mixture of single and double-stranded [^{3}H] *E. coli* DNA and 1 picomole of Cas9 Nuclease, *S. pyogenes* incubated for 4 hours at 37°C releases < 0.1% of the total radioactivity.

Non-Specific DNase Activity (16 hour): A 50 µl reaction in Cas9 Nuclease Reaction Buffer containing 1 µg of λ DNA and 1 picomole of Cas9 Nuclease, *S. pyogenes* incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

Functional Test (Cas9 Nuclease, *S. pyogenes* Targeted Digestion): A 30 µl reaction in 1X Cas9 Nuclease Reaction Buffer containing 1 nM PvuII linearized pBR322 DNA (one targeted site CGCTTGTTTCGGCGTGGGTA), 20 nM SgRNA and 20 nM Cas9 Nuclease, *S. pyogenes* incubated for 1 hour at 37°C results in 95% digestion of the substrate DNA as determined by agarose gel electrophoresis.

Note: 1,000 nM is equal to 159 ng/µl.

(see other side)

CERTIFICATE OF ANALYSIS

Reference:

1. Jinek M. et al. (2012) *Science* 816–821. doi: 10.1126/*Science*.1225829. Epub 2012 Jun 28. PubMed PMID: 22745249.

Reference:

1. Jinek M. et al. (2012) *Science* 816–821. doi: 10.1126/*Science*.1225829. Epub 2012 Jun 28. PubMed PMID: 22745249.

New England Biolabs® is a registered trademark of New England Biolabs, Inc.

Page 2 (M0386)