Cas9 Nuclease, 
S. pyogenes

MO386S
70 pmol 1,000 nM Lot: 0061610
RECOMBINANT Store at –20°C Exp: 10/18

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 with an N-terminal 6X His tag.

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 MgCl₂
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 [³²P]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 CCGTTGGTTCCGGCGTGTTA), 40 nM SqRNA and 20 nM Cas9 Nuclease, S. pyogenes incubated for 1 hour at 37°C results in 90% digestion of the substrate DNA as determined by agarose gel electrophoresis.

Note:
1,000 nM is equal to 159 ng/µl.
Reference: