Cas9 Nuclease, 
*S. pyogenes*

**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 CGGTTGTTCGGCGTGTTGTA), 40 nM SgrRNA 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:** 1000 nM is equal to 159 ng/µl.

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