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Cas9-NLS Nuclease

Cat. Number: GE-5030, GE-5050

Enzyme description:

Cas9-NLS Nuclease is a recombinant Cas9 endonuclease from *Streptococcus pyogenes* fused at the C-terminus with the repeated nuclear localization signal (NLS) of the SV40 virus (PKKKRKV), the size of the protein is 163 kDa. Cas9 Nuclease, in complex with guide RNA (crRNA:tracrRNA duplex) or a single sgRNA, performs site-specific hydrolysis of the phosphodiester bond in double-stranded DNA. The break occurs on the DNA strand between the third and fourth nucleotides from the NGG PAM sequence (Protospacer Adjacent Motif) with the formation of "blunt ends".

Sterilization of the product: enzyme and buffer solutions are sterilized by filtration (pore size 0.2 μm). Analysis of sterility by incubating 50 μl of solutions on Petri dishes with medium for Enterobacterales for 3 days does not reveal bacterial colonies.

Application

Genome editing, CRISPR/Cas9 technology.

Source

Cas9-NLS Nuclease is isolated from an *E. coli* strain containing a plasmid with cloned DNA consisting of the Cas9 gene *Streptococcus pyogenes* and DNA fragments additionally encoding 17 amino acids from the N-terminus and 22 amino acids from the C-terminus enzyme. This construction allows the synthesis of a functional Cas9 nuclease fused to the doubly repeated NLS of the SV40 virus.

Enzyme concentration and packaging: 20 pmol/μl (20 μM).

Cat. No.	Product Name	Quantity	Volume
GE-5030	Cas9-NLS Nuclease	300 pmol	15 μl
GE-5050	Cas9-NLS Nuclease	500 pmol	25 μl

Storage Buffer

50 mM Tris-HCl (pH 7.5 at 25°C), 300 mM NaCl, 0.1 mM EDTA, 1 mM dithiothreitol, 50% glycerol

Quality Control

Each batch of the enzyme is tested for electrophoretic purity in SDS-PAGE, *in vitro* specific activity in the presence of sgRNA, DNase activity (without sgRNA) and sterilization.

Reaction buffer for plasmid DNA hydrolysis *in vitro* (x5)

100 mM HEPES (pH 7.5 at 25°C), 625 mM KCl, 5 mM EDTA, 5 mM dithiothreitol, 30 mM $MgCl_2$, 35% glycerol

Optimum reaction temperature: 37°C.
Enzyme inactivation: incubation for 5 minutes at 65°C.

Storage and transportation conditions
Store at -20°C.
Transportation at temperatures not exceeding +8°C is allowed for up to one day.