Cas9 Nuclease NLS Protein (Lyophilized)

Cat. No. K151

Store at -20°C.

Product Description

The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 system is the latest RNA-guided, endonuclease tool in genome editing which allows for very specific genomic disruption and replacement. The Cas9 nuclease serves to unwind the genomic DNA duplex next to conserved protospacer adjacent motifs (PAMs) and homes in on its target sequence, which is recognized by a complementary single-guide RNA. The resulting double-stranded break gets repaired by the non-homologous end joining (NHEJ) pathway, leading to a disruption in the open reading frame of the targeted gene. Alternatively, by supplying a suitable repair template, virtually any desired point mutation can be introduced at the break point via homology-directed repair (HDR).

The Cas9 nuclease from the bacteria *Streptococcus pyogenes*, abbreviated spCas9, is the most commonly used Cas9 variant. The reason for spCas9 popularity is two-fold. First the spCas9 PAM sequence is 5'-NGG, which is highly abundant in the genome allowing virtually any gene to be targeted. The spCas9 enzyme also has on average a higher efficiency *in vivo* compared to other variants. Cas9 Nuclease NLS contains a SV40 T antigen nuclear localization sequence (NLS) on the C-terminus of the protein.

Product Component	Quantity	Part No.
Cas9 Nuclease NLS Protein	200 µg (1.25 nmol)	K151-1
10X Cas9 Reaction Buffer	1.25 ml	K000

Preparation Note

To resuspend the lyophilized Cas9 protein to a concentration of 10 µM (1.6 µg/µl) add 125 µl nuclease-free H_2O to K151 and pipette gently. The solution should be incubated at room temperature for 10 minutes and centrifuged before first-time use.

Protocol

In vitro digestion of DNA

1. Add the following components to a sterile, nuclease-free tube sitting on ice:

Product Component	Volume
sgRNA (300 nM)	3 µl
Cas9 Nuclease NLS Protein (1 µM)[1]	1µl
10X Cas9 Reaction Buffer	3 µl
Nuclease-free H_2O	20 µl
Pre-incubate for 15 minutes at 37°C	
Substrate DNA (30 nM)	3 µl

[1] Dilute to 1 µM. See General Notes for further details.

2. Collect all components by a brief centrifugation. Incubate the reaction at 37°C for 1 hour.
3. Analyze fragments via agarose gel electrophoresis.

General Notes

- Dilute Cas9 Nuclease NLS Protein (10 µM) to 1 µM using the following:
  - **10X Cas9 Reaction Buffer** for immediate use.
  - **10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 300 mM NaCl, and 50% (v/v) Glycerol** if storing in -20°C before use.
- The substrate DNA : sgRNA : Cas9 molar ratio must be kept at 1:10:10 for highest efficiency.

Applied Biological Materials Inc.● 1-866-757-2414 ● info@abmGood.com ● www.abmGood.com