Datasheet for ABIN3188219

Cas9 Nickase D10A NLS Protein

Overview

Quantity:	8 µg
Application:	Genome Editing with Engineered Nucleases (GEEN)

Product Details

Characteristics:	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. One concern with the current CRISPR Cas9 technology is the potential off-target effects of the Cas9 nuclease activity. To improve the off-target mutagenic effects of this system, the Cas9 Nickase D10A NLS Protein was developed with a D10A mutation in its RuvC1 nuclease domain with an SV40 T antigen nuclear localization sequence (NLS) on the C-terminus of the protein. This mutant form results in the generation of single strand nick instead of a double stranded break (DSB), like that generated by the Cas9 Nuclease, at the target site. Since a single strand break, or nick, is normally quickly repaired through the homology directed repair pathway using the intact complementary DNA strand as the repair template, off-target effects of the Cas9 Nickase is minimized. To utilize Cas9 Nickase for genome editing, two gRNAs, instead of one is required. The two gRNAs will be designed on opposite DNA strands but with close proximity to ensure that a DSB is induced once the two strands are nicked by the Cas9 Nickase. This paired Cas9 Nickase modification reduces off-target effects because the two gRNAs need to work together to produce a DSB. Once the DSB is created, either the NHEJ or HDR pathway will be activated to complete the genome editing process. The Cas9 Nickase can also be used to create nucleotide modifications by homologous recombination if a repair template DNA containing the desired modification is introduced along with the gRNA and Cas9 nickase.
Components:	Enzyme supplied with 10X Reaction Buffer

Application Details

Restrictions:	For Research Use only

Handling

Concentration:	1000 nM
Buffer:	10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 300 mM NaCl, and 50 % (v/v) Glycerol.
Storage:	-20 °C

Publications

Product cited in:	Johnson, Drugan, Miller, Evans: "38" in: , Vol. 1363, Issue Nucleic acids research, pp. 28-39, (1991)