



Cas9 Nickase H840A NLS Protein

Store at -20 °C

Cat. No.	Description	Concentration	Quantity
K038	Cas9 Nickase H840A NLS Protein	1000 nM	50 pmol (50 µl)
K138	Cas9 Nickase H840A NLS Protein	10 µM	250 pmol (25 µl)

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. One concern with the current CRISPR Cas9 technology is the potential off-target effects of the Cas9 nuclease.

To improve the off-target mutagenic effects of this system, the Cas9 Nickase H840A Protein was developed with a H840A mutation in its HNH-like nuclease domain. Unlike the Cas9 nuclease, this mutant form generates a single-stranded nick instead of a double-strand break (DSB). Because a single DNA nick is quickly repaired with high fidelity by the cellular machinery, the system requires two closely juxtaposed nicks in order to trigger the same genomic disruption as the Cas9 nuclease. This effectively boosts the recognition sequence to 40 instead of 20 nucleotides, and, as a result, off-target effects become highly unlikely. Thus, the double-nickase CRISPR system offers unparalleled specificity to satisfy even the most stringent of experimental requirements.

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 H840 Nickase NLS contains a SV40 T antigen nuclear localization sequence (NLS) on the C-terminus of the protein.

Kit Components

Part No.	Product Components	50 pmol
K038	Cas9 Nickase H840A NLS Protein	50 µl
K000	10X Cas9 Reaction Buffer	1.25 mL
Part No.	Product Components	250 pmol
K138	Cas9 Nickase H840A NLS Protein	25 µl
K000	10X Cas9 Reaction Buffer	1.25 mL

Product Source

Recombinant *E. coli*.

Storage Conditions

Store all components at -20 °C. Avoid repeated freeze-thaw cycles of all components to retain maximum performance. All components are stable for 1 year from the date of shipping when stored and handled properly.

Enzyme Storage Buffer

10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 300 mM NaCl, and 50% (v/v) Glycerol.

10X Cas9 Reaction Buffer Components

200 mM HEPES, 50 mM MgCl₂, 1 M NaCl, 1 mM EDTA, pH 6.5.

Reaction Conditions

Use 1X Cas9 Reaction Buffer and incubate at 37 °C.