Tribioscience

Cas9 D10A Nickase GFP NLS Protein

Catalog	Unit
TBP0203	47 µg

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 counteract off-target mutagenic effects of this system, the Cas9 Nickase D10A was developed with a D10A mutation in its RuvC1 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 from the bacteria Streptococcus pyogenes, abbreviated spCas9, is the most commonly used Cas9 variant. The fusion of Cas9 to GFP allows for visual confirmation of transfection as well as subsequent verification of Cas9 clearance from the cells. Cas9 Nickase D10A GFP can also be used for FACS applications and screening. Cas9 Nickase D10A GFP NLS contains a SV40 T antigen nuclear localization sequence (NLS) on the C-terminus of the protein.

Component

Product Component	Quantity
Cas9 Nickase D10A GFP NLS Protein	25 µl (250 pmol, 10 µM)
10X Cas9 Reaction Buffer	1.25 ml

Store at -20°C.

Protocol

In vitro digestion of DNA

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

Component	Volume
sgRNA #1 (300 nM)	3 µl
sgRNA #2 (300 nM)	3 µl
Cas9 Nickase D10A 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 Nickase D10A GFP 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.

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