Tribioscience

Cas9 Nuclease Protein

Catalog	Unit
TBP0191	40 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.

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 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.

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.

Component

Product Component	Quantity
Cas9 Nuclease 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 (300 nM)	3 µl
Cas9 Nuclease 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 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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