Optimized Cas9 Nuclease - S. pyogenes

CRISPR/Cas9 Delivery Kit: Cas9 + Pro-DeliverIN CRISPR transfection reagent

Product information

Size: $C_{21}H_{19}N_3O_7S_2$

Isoelectric point: 9.26

Concentration: 1 mg/mL in Hepes 10 mM pH 7.5, 250 mM NaCl, 1mM DTT, 50% Glycerol

100 µg of Cas9 nuclease = 608 pmol

Cas9 nuclease derived from Streptococcus pyogenes. Contains a N-Terminal His Tag and 2 Optimized Nuclear Localization Sequences (NLS) – 1 N-terminal NLS and 1 C-terminal NLS.
Cas9 protein was produced in BL21(DE3) *E. coli*. After centrifugation, cultures were lysed by sonication and fraction were separated by another round of centrifugation. The first purification step was performed using His Trap column and was followed by a desalting procedure on desalting column. Protein was finally purified by gel filtration column.

Efficient nucleic acid delivery represents a **critical step for genome editing** experiments. For the most efficient Cas9 nuclease delivery, we recommend **Pro-DeliverIN CRISPR Transfection Reagent.**

Description

Optimized **Cas9 Nuclease** *S. Pyogenes* is designed for genome editing in living cells or organisms and for *in vitro* digestion.

Why choose Cas9 Protein instead of Cas9 DNA or mRNA?
The Cas 9 recombinant protein is delivered more rapidly than nucleic acid and is fully active once inside the cells without latency period (in contrast to transcription and translation machinery required for the nucleic acids).
These features make nuclease protein delivery particularly well suited for precision genome engineering.

Efficient nucleic acid delivery represents a **critical step for genome editing** experiments. For the most efficient Cas9 nuclease delivery, we recommend **Pro-DeliverIN CRISPR** Transfection Reagent.

Applications

Target cleavage by Cas9 requires specific single guide RNA (sgRNA), resulting from the association of a CRISPR RNA (crRNA) that provides sequence specificity and a trans-activating RNA (tracrRNA) that allows docking to the Cas9 nuclease. Thus, sgRNA must present sequence specificity and binding capacity to nuclease. Choose wisely your sgRNA sequence to avoid undesired effects due to mismatches.

For sgRNA design: you can refer to the open source CRISPR Design tool from the MIT: http://crispr.mit.edu/

Use, handling and storage

For Research Use Only. Not for use in humans. Not for use in diagnostic or therapeutic purposes.

Shipping conditions: Dry ice.

Storage conditions: Cas9 nuclease and buffer: -20°C.

Shelf life: 1 year from the date of purchase.

⚠ Avoid freeze/thawing cycles.

Kit contents

CAS9050: 50 µg Cas9 nuclease in 50 µL.
CAS9100: 100 µg Cas9 nuclease in 100 µL.
CAS9500: 500 µg (5x100 µg) Cas9 nuclease in 500µL.
Reagent supplied: Cas9 reaction buffer (10X)

Special CRISPR/Cas9 Delivery Kit
CAS9PIC: 50 µg Cas9 nuclease + 100 µL of Pro-DeliverIN CRISPR

Certificate of analysis on demand.

Related Products

Ref	Description
#PIC60500	Pro-DeliverIN CRISPR
#MRNA27-100	Cas13d mRNA
#MRNA30-100	Cas9 mRNA

Purchaser Notification | Conditions of Sale

This product is sold in accordance with our general conditions of sale that you can find on our website: https://ozbiosciences.com/content/3-terms-and-conditions.

Rev 06/25 CS

Protocols

In vitro digestion

This protocol is given for digestion of linearized or double-stranded plasmid *in vitro* using sgRNA and Cas9 protein.

NOTE: it is recommended to test sgRNA performance before Cas9 delivery for genome editing.

A. *sgRNA preparation* (not included in the kit): we recommend preparing a stock solution of **3 µM** duplex concentration.

1. Prepare solutions of crRNA and tracrRNA at **100 µM** in **10 mM** Tris **pH 7.4**
2. Add **3 µL** each to **94 µL** of a solution of **10 mM** Tris, **pH 7.4**
3. Incubate **5 min** at **95 °C**
4. Allow to cool down to room temperature.

B. *In vitro target sequence cleavage* with recombinant Cas9 and synthesized sgRNA:

Components	Volume
Nuclease free water (final 30 µL)	24 µL
10X Cas9 reaction buffer	3 µL
sgRNA (100 nM final)	1 µL
Cas9 protein	1 µL (1 µg)
	Incubation 10 min x RT
DNA*	1 µL (1 µg)
	Incubation 1 h x 37°C

* We recommend using a DNA solution at 1 mg/mL in TE.

Then, analyze cleavage product by electrophoresis on agarose gel (see figure 1 below):

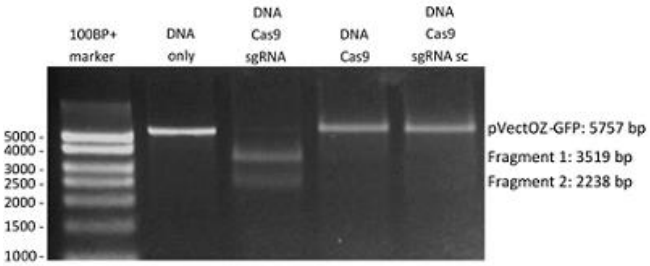

Figure 1. Cas9 nuclease S. Pyogenes is used to cleave pVectOZ-GFP plasmid in vitro. pVectOZ-GFP was linearized using XhoI restriction enzyme and DNA was incubated in presence of Cas9 + sgRNA targeting GFP, Cas9 alone or Cas9 + sgRNA scramble (sc). In presence of targeting sgRNA, linearized DNA is cleaved in two fragments.

Genome editing in cells using Pro-DeliverIN CRISPR

Pro-DeliverIN CRISPR (#PIC60100 or #PIC60500) can be used to deliver Cas9 nuclease directly into living cells in culture or organisms using.

The protocol below is an example for genome editing in a 24-well plate:

1. Prepare a mix of **1 µg** of Cas9 nuclease and **250 ng** of your sgRNA
2. Mix gently by pipetting up and down
3. Incubate **10 min** at **RT** (recommended 25°C) to allow formation of Cas9/RNPs complexes
4. Directly add **2 µL** of Pro-DeliverIN CRISPR
5. Incubate **20 min** at **RT** (recommended 25°C) to allow formation of complexes
6. Complement to **50 µL** with complete culture medium
7. Add complexes dropwise onto cells and incubate under standard culture conditions.

For more information, please refer to **Pro-DeliverIN CRISPR** protocol or send an email to: tech@ozbiosciences.com.

Purchaser Notification | Conditions of Sale

This product is sold in accordance with our general conditions of sale that you can find on our website: https://ozbiosciences.com/content/3-terms-and-conditions.