Optimized Cas9 Nuclease - *S. pyogenes*
CRISPR/Cas9 Delivery Kit: Cas9 + Pro-DeliverIN CRISPR transfection reagent

Description

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

Characteristics

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. A first purification step was performed using HisTrap column and was followed by a desalting procedure on desalting column. Protein was finally purified by gel filtration column.

- Size: 164.48 kDa
- Isoelectric point: 9.26
- Concentration: 1 mg/mL
  Supplied in Hepes 10 mM pH 7.5, 250 mM NaCl, 1mM DTT, 50% Glycerol
- 100 µg of Cas9 nuclease = 608 pmol

Sizes

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 + 100µL of ProDeliverIN CRISPR

Storage & Shipping

Cas9 nuclease & Cas9 reaction buffer must be stored at -20°C. Avoid freeze/thawing cycles; we recommend to aliquot the Cas9 nuclease solution for a better storage.

Shipping condition: Dry Ice

Applications

IMPORTANT/PREREQUISITE: Target cleavage by Cas9 requires specific sgRNA (single guide RNA), resulting from the association of a crRNA (CRISPR RNA) that provides sequence specificity and a tracrRNA (trans-activating RNA) that allows docking to the Cas9 nuclease. Thus, sgRNA bears two specificities: sequence specificity and binding capacity to nuclease. This is why we recommend choosing wisely your sgRNA sequence to avoid undesired effects due to mismatches.

For sgRNA design: we recommend using the open source CRISPR Design tool from the MIT: http://crispr.mit.edu/

In vitro digestion

Recommended to test sgRNA performance before Cas9 delivery for genome editing.
This protocol is given for digestion of linearized or double-stranded plasmid in vitro using sgRNA and Cas9 protein.

I/sgRNA preparation (not included in the kit): we recommend to preparing stock solution of 3µM duplex concentration.

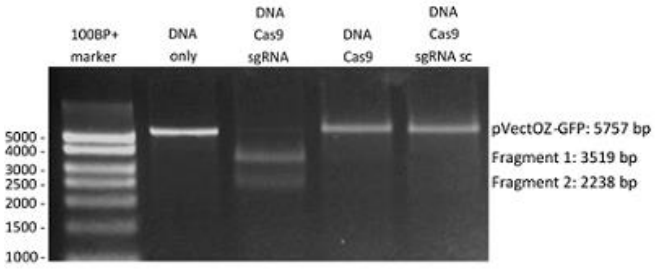
1. Prepare solutions of crRNA and tracrRNA at 100 µM in 10 mM Tris pH 7.4
2. Add 3 µL each to 94 µL of a solution of 10 mM Tris, pH 7.4
3. Incubate 5 min at 95 °C
4. Allow to cool down to room temperature.

II/ In vitro target sequence cleavage with recombinant Cas9 and synthesized sgRNA

Components	volume
Nuclease free water (final 30µL)	24 µL
10X Cas9 reaction buffer	3 µL
sgRNA (100nM 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 1mg/mL in TE.

Then, analyse cleavage product by electrophoresis on agarose gel (see results below):



Cas9 nuclease S. Pyogenes is used to cleave pVectOZ-GFP plasmid in vitro. pVectOZ-GFP was linearized using xhol 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

Cas9 nuclease can be delivered directly into living cells in culture or organisms using ProDeliverIN CRISPR (# PIC60100 or #PIC60500). 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 ProDeliverIN 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 **ProDeliverIN CRISPR** protocol or send an email to: tech@ozbiosciences.com

Additional products for CRISPR Cas9 experiments:

- **PolyMag CRISPR** for Genome editing using expression plasmids
- **RmesFect CRISPR** for mRNA transfection
- **ViroMag CRISPR** to enhance transduction efficiency of CRISPR/Cas9 viruses

Contact Us

Feel free to contact us for all complementary information and remember to visit our website to stay informed on the latest breakthrough technologies and updated on our complete product list. (www.ozbiosciences.com)
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