CRISPR - Cas9 Nuclease (S. Pyogenes); CRISPR-associated Cas-System

Description
Cas9 Nuclease is the purified recombinant Streptococcus pyogenes Cas9 enzyme containing a nuclear localization signal (NLS) at the C-terminal for targeting to the nucleus. This enzyme is designed to perform CRISPR/Cas9-mediated genome editing . The physical purity of this enzyme is ≥98% as assessed by SDS-PAGE with Coomassie® blue staining.

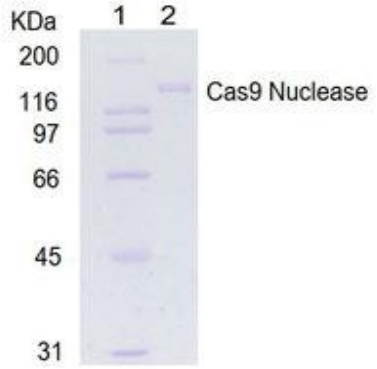

Fig. 2: Lane 1. Protein Marker
Lane 2. Cas9 Nuclease

Product Source:

E. coli BL21 (DE3) strain expressing a Cas9 gene from Streptococcus pyogenes with an N-terminal 6xHis tag and C-terminal SV40 nuclear localization signal (NLS).

Content:

Cas9 Nuclease in: 50 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, pH 7.5 @ 25 ºC

Concentration:

Standard-version: 160 ng/µl
HC-Version: 1600 ng/µl

10x Cas9 Nuclease Reaction Buffer (200 mM HEPES, 1000 mM NaCl, 50 mM MgCl2, 1 mM EDTA, pH 6.5 @ 25 °C)
Storage: at -20°C for 24 months, avoid frequent thawing and freezing

Transport: with blue ice

Standard Protocol	
1) Target DNA	x µl - approx 100 ng
sgRNA	x µl - approx. 4000 ng
10x Cas9 Reaction Buffer	3 µl
Cas9 Nuclease	1 µl - approx. 160 ng
Water up to	30 µl

2) Gently mix the reaction mixture and centrifuge briefly.

3) Incubate at 37 ºC for 60 min.

4) Add 1 µl RNase (4 mg/ml)

5) Incubate at 37 ºC for 20 min.

6) Run 0.7 to1% agarose TBE gel.

Recomended Transfection reagents (not provided):

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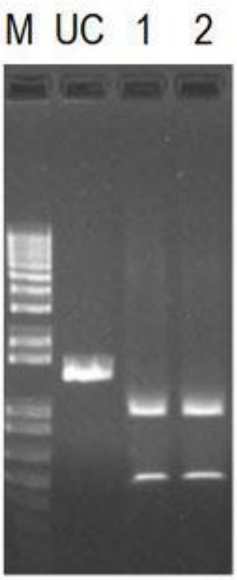
- Nucleofector™ Kits from Lonza
- Lipofectamine™ CRISPRMAX™ from Thermo Fisher
- Electroporation of mammalian cells with Cas9-sgRNA ribonucleoprotein complexes. Any electroporation machine can be used.

Cas9 Nuclease functional testing was done by in vitro DNA cleavage assay with the following protocol which gives more than 95% digestion of the substrate DNA as determined by agarose gel electrophoresis.



M: Marker

UC: Uncut,

1: 1 µl Cas9,

2: 2 µl Cas9

QC-Assay:
Cas9 nuclease is free from detectable RNase, Endonuclease (nicking) and non-specific DNase activities.

Ordering Information:

Cat.-no	Description	Amount
310	Cas9 Nuclease 2x40 µg (160 ng/µl)	2x250 pmol
312HC	Cas9 Nuclease high-concentration (1600 ng/µl)	500 pmol
314HC	Cas9 Nuclease high-concentration (1600 ng/µl)	2500 pmol

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