

## Cas9 Nuclease protein NLS

**Cat. No.** C29010001

**Description:** Full length recombinant *Streptococcus pyogenes* Cas9 Nuclease, with an N-terminal and C-terminal Nuclear Localization Signal (NLS), produced in *E. coli*.

The Cas9 Nuclease comes with a 10x Reaction Buffer (10x H buffer, 1.0 ml: 500 mM Tris-HCl pH 7.9, 1 M NaCl, 100 mM MgCl<sub>2</sub>, 10 mM DTT).

**Source:** *E. coli*

**Lot #:** 001

**Size:** 75 µg/ 25 µl

**Concentration:** 3 µg/µl

**Specificity:** *Streptococcus pyogenes*

**Precautions:** This product is for research use only. Not for use in diagnostic or therapeutic procedures.

**Purity:** Affinity purified using Ni-based resins, >95% purity as determined by SDS-PAGE

Low endotoxin (<1 EU/µg)

DNase/RNase: No significant changes of the electrophoretic pattern are observed after the incubation of 10 µg of the protein with 1 µg of DNA/RNA substrate for 1 hour at 37°C.

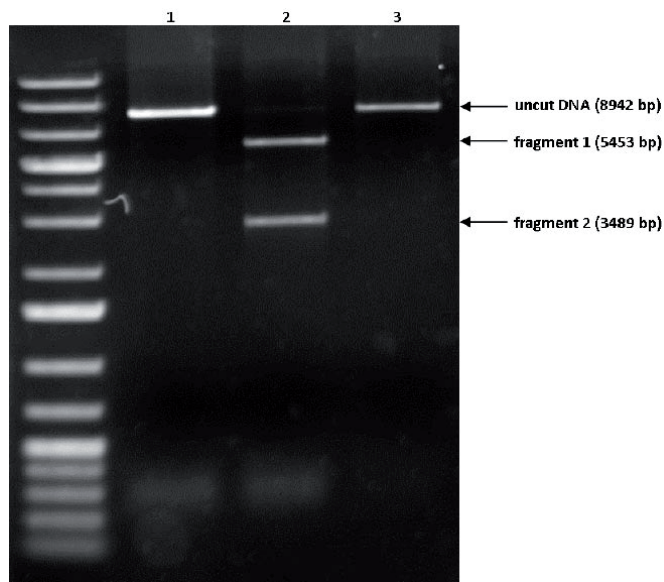
**Storage:** Store at -20°C; guaranteed stable for 2 years from date of receipt when stored properly.

**Storage buffer:** 10 mM Tris-HCl (pH 7.5 at 25°C), 300 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 50% Glycerol.

### Protein description

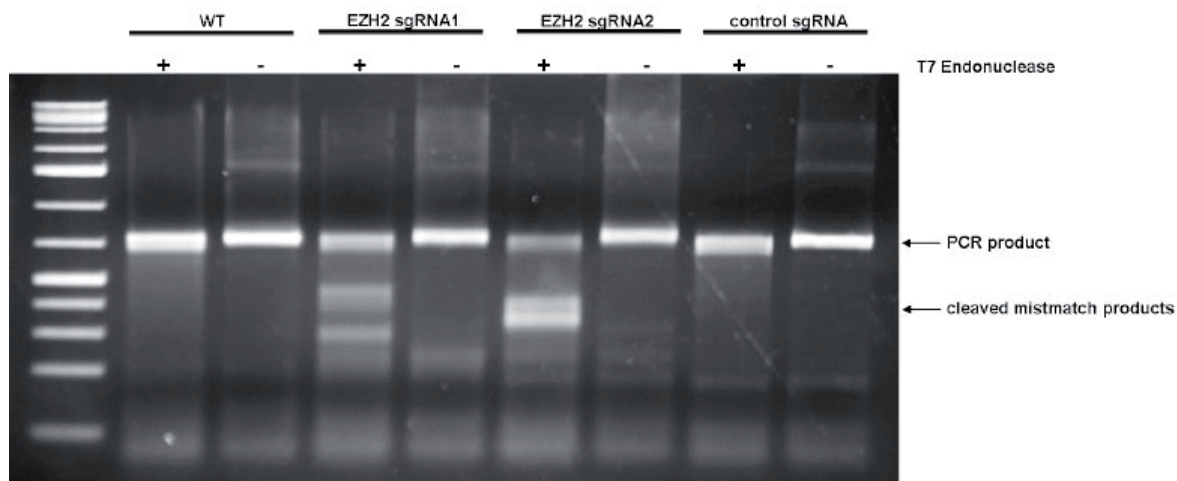
The CRISPR/Cas genome editing system consists of two components: a "guide" RNA (gRNA) and the Cas9 endonuclease. The gRNA is a short synthetic RNA composed of a "scaffold" sequence necessary for Cas9-binding and a user-defined ~20 nucleotides "targeting" sequence which defines the genomic target to be modified. Cas9 protein and the gRNA form a very stable ribonucleoprotein complex through interactions between the gRNA scaffold domain and surface-exposed positively-charged grooves on Cas9. Recombinant Cas9-NLS protein complexed with in vitro transcribed guide RNAs cleaves the target sequence and is a powerful and precise tool to edit the genome of eukaryotes.

## Quality control



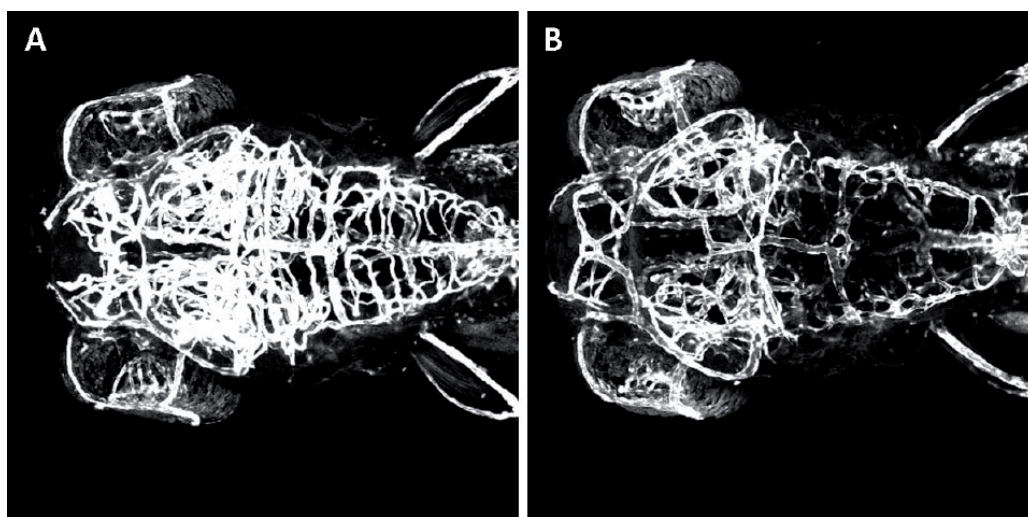
**Figure 1. In vitro DNA cleavage assay using the Diagenode Cas9 Nuclease protein NLS**

A Cas9 reporter plasmid linearized with PvuI was incubated with (lanes 2 and 3) or without (lane 1) the Diagenode Cas9 NLS protein (Cat. No. C29010001) for 1 hour at 37°C. Lane 1 shows the untreated plasmid. A negative control reaction that lacked sgRNA is shown in lane 3. Cleavage efficiency was assessed by agarose gel electrophoresis.



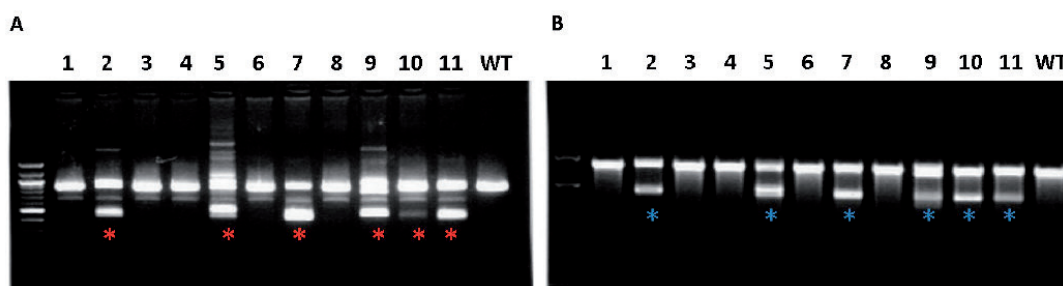
**Figure 2. In vitro transfection assay using the Diagenode Cas9 Nuclease protein NLS**

HEK293T cells were transfected with the Diagenode Cas9 protein NLS (Cat. No. C29010001) and two different sgRNAs targeting EZH2. Untransfected cells (WT) and cells transfected with a non-targeting sgRNA (control sgRNA) were used as negative controls. PCR was performed on genomic DNA from the cells with primers flanking the CRISPR targeting site (700 bp amplicon) using the MethylTaq DNA polymerase (C09010010). The PCR products were tested for CRISPR/Cas9 induced mutations by a T7 Endonuclease I assay. Cleavage at heteroduplex mismatch sites was assessed by agarose gel electrophoresis. These results show that the Cas9 Nuclease Protein NLS, when combined with specific sgRNAs provides consistent and effective gene editing.



**Figure 3. Efficient mutagenesis with the Diagenode Cas9 Nuclease protein NLS**

Zebrafish embryos at the one-cell stage were injected with the Diagenode Cas9 Nuclease protein NLS (Cat. No. C29010001, 300pg) and an sgRNA (30pg) RNP complex, targeting a gene required for angiogenesis in the brain. Figure 3B shows a confocal z-stack of the cranial vasculature of Tg[kdrl:GFP] at 4dpf in dorsal view (anterior to the left). An uninjected control sibling is shown in figure 3A. On average, 85% of the injected embryos display a total absence of hindbrain intracerebral blood vessels at 4dpf. This knock-out causes specific CNS vascular defects showing that the gene of interest is inactivated by the CRISPR/Cas9 system.



**Figure 4. Generation of knock-in mice using the Diagenode Cas9 Nuclease Protein NLS**

Fertilized mouse eggs (C57BL/6N) were injected with the Diagenode Cas9 Nuclease protein NLS (Cat. No. C29010001) single-stranded oligonucleotides, used as a Homology Directed Repair (HDR) template to edit the mouse Smpd3 locus and a guide RNA. The template incorporates a BamHI restriction site for genotyping. Two-cell stage embryos were transferred to a foster-mother and 11 neonatal mice were analyzed. Figure 4A shows a mismatch detection assay using a surveyor assay (Cel1 digest of PCR products). Positive candidates (CEL1-cleaved DNA) are marked with a red \*. Figure 4B shows a BamHI digestion of the PCR products indicating that the donor DNA has been integrated in the genome and specific sequence changes have been introduced. Positive candidates (BamHI-cleaved) are marked with a blue \*. More details are shown in the table below.

Material	Final concentration	Injected sites	Number of embryos injected	Number of embryos transferred	Number of neonates	KOs	KIs
Cas9 Nuclease	30 ng/μl	Cytoplasm + Pronucleus	123	114	11	6/11 (54.5 %)	6/11 (54.5 %)
sgRNA	50 ng/μl						
ssODN	100 ng/μl						

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