Miltenyi Biotec

StemMACS™ Cas9 Nuclease mRNA
human

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1. Description

Components	**20 µg StemMACS™ Cas9 Nuclease mRNA** encoding Cas9 (CRISPR associated protein 9), a RNA-guided DNA endonuclease from *Streptococcus pyogenes* A20. The coding sequence has been modified with N- and C-terminal nuclear localization signals (NLS) for efficient transport of the Cas9 protein into the nucleus [1]. **1 mL Double-distilled Water,** RNase-free
Specifications	*In vitro* transcribed, polyadenylated and capped mRNA that has been modified with pseudouridine and 5-methyl-cytidine to reduce the innate antiviral response to single-stranded RNA.
Formulation	Lyophilized from a filtered (0.2 µm) solution.
Storage	Store the lyophilized product at –20 °C. The expiration date is indicated on the label. After reconstitution, the product can be stored at –70 °C for up to 3 month.
Quality control	mRNA size has been verified on an Agilent Bioanalyzer System. Cas9 protein expression and function after transfection was confirmed by eGFP gene knock out in mouse ES cells.

1.1 Principle

The transient expression of key developmental regulators, recombinases or markers via mRNA transfection is a powerful tool for modulating cell fate. StemMACS mRNAs are highly pure, *in vitro*-transcribed mRNAs that have been carefully optimized and validated to ensure high level expression after transfection.

1.2 Background information

Cas9 originates from the CRISPR (clustered regularly interspersed palindromic repeats) adaptive immunity system of *Streptococcus pyogenes*.
Cas9 nuclease can be used to introduce site-directed double strand breaks into the genomic DNA. These will subsequently be repaired through one of two repair pathways: non-homologous end joining (NHEJ) or homology directed repair (HDR). NHEJ repair is error prone and will frequently result in nucleotide deletions or insertions (indel). If such insertions/deletions result in a frame shift or introduction of a premature stop codon, they will disrupt the open reading frame of the target gene. Therefore, this approach can be used to generate a gene knock out.
Alternatively, the HDR pathway can be used to introduce specific changes into the gene of interest. By providing a DNA repair template that contains the desired modification flanked by regions that are homologous to the genomic target sequence, HDR enables genomic integration of the edited target sequence.
The position of the Cas9 cleavage site is determined by two factors: the target sequence recognized by the guide RNA and the presence of a protospacer adjacent motif (PAM, in the case of *Streptococcus pyogenes* Cas9 the nucleotide sequence NGG). The Cas9/guide RNA complex will recognize the conjunction of both sequence elements and generate a double strand break 3 base pairs upstream of the PAM[2].

1.3 Applications

- Site-specific gene knockout
- Gene editing by homologous recombination

2. Protocol: Reconstitution of lyophilizate

▲ RNA is susceptible to degradation by exogenous ribonucleases. Wear gloves, use RNase-free reagents, tubes, and pipette tips.

1. Dissolve StemMACS Cas9 Nuclease mRNA in 200 µL of Double-distilled Water. Vortex thoroughly. The final concentration will be 0.1 µg/µL.
2. Briefly centrifuge to collect the content at the bottom of the tube.
3. Prepare aliquots and store at –70 °C to –80 °C. Do not subject aliquots to more than two freeze-thaw cycles.

For satisfactory transfection results, use a protocol that is optimized for your specific cell type. StemMACS eGFP mRNA or StemMACS Nuclear eGFP mRNA allow easy evaluation of transfection efficiency and are recommended as positive controls.

Miltenyi Biotec B.V. & Co. KG
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macsde@miltenyi.com
www.miltenyibiotec.com

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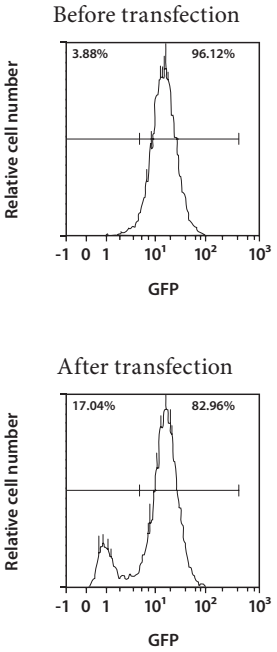
3. Example

HM1 mouse embryonic stem cells carrying a GFP reporter gene were transfected with 750 ng StemMACS Cas9 Nuclease mRNA and 250 µg plasmid encoding the guide RNA using Lipofectamine® 2000. Cells were analyzed by flow cytometry 96 hours after transfection. 17.04% of all cells were GFP-negative indicating successful eGFP knockout.
Transfection efficiency was >90% as determined by independent electroporation of mCherry mRNA into control HM1 cells (not shown).



4. References

1. Cong, L. *et al.* (2013) Multiplex genome engineering using CRISPR/Cas systems. Science 339 (6121): 819–823.
2. Jinek, M. *et al.* (2012) A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science 337 (6096): 816–821.

Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com/local to find your nearest Miltenyi Biotec contact.

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