Miltenyi Biotec

StemMACS™ Cas9 Nickase mRNA
human

Order no. 130-107-679

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1. Description

Components	**20 µg StemMACS™ Cas9 Nickase mRNA** encoding a D10A mutated version of 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 nickase function after transfection was confirmed by eGFP gene knock out using a pair of guide RNAs.

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 in *Streptococcus pyogenes*. The original Cas9 nuclease has two catalytic domains, the RuvC-like nuclease domain that cleaves the non-complementary strand and the HNH nuclease domain that cleaves the complementary strand of DNA[2]. Cas9 nickase carries a D10A point mutation that eliminates the nuclease function of the RuvC-like domain, so that only the strand of the DNA that is complementary to the guide RNA sequence is cleaved.

Cas9 nickase is used to induce site-directed single strand breaks within the genomic target DNA. By introducing a sequence of interest linked to homologous sequences of the target region, single strand break repair enhances homologous recombination, and genomic integration of the sequence of interest. In contrast to Cas9 nuclease, non-homologous end joining (NHEJ) is not involved in the repair of single strand breaks introduced by Cas9 nickase. Therefore, potential off-target single strand breaks will be reliably repaired by the base excision repair (BER) pathway without the risk of offside genomic modifications.

A pair of guide RNAs can be used to introduce two single strand breaks. Using two guide RNAs designed to recognize the opposite strands with less than 8 bp overlap between the guide RNA sequences and to give 5′-overhangs has been shown to efficiently induce insertions/deletions (indel) by non-homologous end joining (NHEJ)[3]. Thereby, specificity of gene knock outs can be dramatically improved[4].

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 Nickase 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.

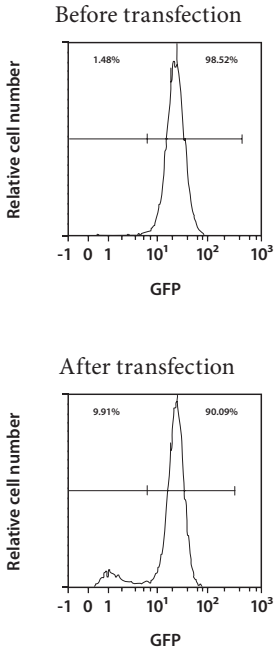
For satisfactory transfection results, use a protocol that is optimized for your specific cell type. StemMACS eGFP mRNA or StemMACS Nuclear eGFP mRNA allow easy evaluation of transfection efficiency and are recommended as positive controls.

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3. Example

HM1 mouse embryonic stem cells carrying a GFP reporter gene were transfected with 750 ng StemMACS Cas9 Nickase mRNA and two plasmids encoding guide RNAs (125 ng each) using Lipofectamine® 2000. Cells were analyzed by flow cytometry 72 hours after transfection. 9.91% 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.
3. Ran, F. A. *et al.* (2013) Double nicking by RNA-guided CRISPR Cas9 for enhanced genome editing specificity. Cell 154 (6): 1380–1389.
4. Mali, P. *et al.* (2013) CAS9 transcriptional activators for target specificity screening and paired nickases for cooperative genome engineering. Nat. Biotechnol. 31 (9): 833–838.

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