

Description

Ready-to-use stabilized Cas9 mRNA

Cap Modification: Cap 1 | **Poly (A) Tail:** Yes

Concentration: 1.0 mg/mL

Buffer: 1 mM Sodium Citrate, pH 6.4

Full length mRNA: 4459 nt

Molecular weights: #MRNA30: 1444890 g/mol; #MRNA31:

1461600 g/mol; #MRNA25: 1453245 g/mol

Cas 9 mRNAs encode the CRISPR associated (Cas) protein 9 (Cas9) protein of the *Streptococcus pyogenes* with an N and C terminal nuclear localization signal (NLS), and a HA-Tag on the 3' end. OZB mRNAs are produced by *in vitro* transcription. mRNAs are stabilized at the 5' end by modified nucleotides capping (Cap1) and contain a poly(A) tail at the 3' end. Sequences have been optimized to yield improved stability and performance. Cas9 mRNA #MRNA30 does not bear any additional nucleotide modifications while #MRNA31 is modified with 5-methoxyuridine (5moU), #MRNA25 is modified with N1-methyl-pseudouridine (N1-mψ) to reduce innate immune response.

Applications

The mRNA Cas9 encodes for the RNA-guided Cas9 endonuclease used to induce site-directed double strand breaks in DNA. These breaks can lead to gene inactivation or introduction of heterologous genes through non-homologous end joining and homologous recombination respectively, providing efficient tool for Genome Editing¹⁻⁴.

1. Cong L. *et al.*, Science, 2013. DOI:10.1126/science.1231143.
2. Mali P., *et al.*, Science, 2013. DOI:10.1126/science.1232033.
3. Jinek M., *et al.*, Science, 2012. DOI:10.1126/science.1225829.
4. Cho SW., *et al.*, Nat Biotechnol., 2013. DOI:10.1038/nbt.2507.

General considerations on OZB's mRNA

Cas9 mRNAs resemble fully matured mRNAs with 5' cap1 structure and 3' polyA tail, therefore ready to be translated by the ribosome. mRNA transfection provides several advantages over plasmid DNA (pDNA) delivery. It does not require nuclear uptake for being expressed since translation of mRNA occurs directly into cytoplasm. Indeed, nuclear delivery (transport through nuclear membrane) is one of the principal barriers for transfecting slow or non-dividing cells and consequently, mRNA transfection is particularly attractive for such purpose. This approach presents also the advantage of being non-integrative which is particularly appealing for stem cells, regenerative medicine or vaccine fields. Contrary to pDNA, mRNA cannot lead to genetic insertion causing mutations. Moreover, the protein expression from the mRNA is promoter-independent and faster than with DNA. For transfection we recommend RmesFect™ (#RM21000) and RmesFect™ Stem (#RS31000).

Quality Controls

Items	Specification	Standard QC	Superior Grade QC*
Integrity	Agarose gel mobility and HPLC	✓	✓
Concentration	1mg/ml +/- 5%	✓	✓
A260/280	>1.8 for Unmodified mRNAs >1.7 for chemically modified mRNAs	✓	✓
Sterility	Absence of bacterial growth at 37°C	✓	✓
Functionality**	Test for protein expression	✓	✓
Endotoxin	<0.5 EU/mL		✓
dsRNA	<0.5%		✓

* Our catalogue mRNAs undergo the standard QC. Superior Grade QC can be performed as an additional prestation.

** For reporter mRNAs and Spike SARS-Cov2 related mRNAs only for catalog mRNAs. Can be included in superior Grade QC

Certificate of analysis on demand.

Use, handling and storage

For Research Use Only. Not for use in humans. Not for use in diagnostic or therapeutic purposes.

Long term storage (months): -80°C.

Short term storage (few days): -20°

We recommend to aliquot the mRNA solution for a better storage. Follow good laboratory practices for mRNA handling (work on ice, avoid freeze/thaw cycles, do not vortex, use RNase free water and barrier tips, ...)

mRNA Stability

RNA can suffer degradation when not handled, stored, or used properly. In order to assess the stability of OZ Biosciences mRNAs, we have tested a randomly chosen RNA from our catalog and submitted it to several freeze/thaw cycles as well as a 15-day storage at room temperature (RT). mRNA did not show any sign of degradation in any condition as observed on agarose gel (cf Stability note available on our website).

Kit contents

Cas9 mRNAs-20: 20 µg of mRNA.
Cas9 mRNAs-100: 100 µg mRNA.
Cas9 mRNAs-1000: 1 mg of mRNA.

Related Products

Ref	Description
#RM20500/21000	RmesFect™ transfection reagent (mRNA)
#RS30500/31000	RmesFect™ Stem transfection reagent (mRNA)
#MRNA11/15/22	mRNA GFP unmodified or 5moU or N1-mpU
#MRNA12/16/24	mRNA LUC unmodified or 5moU or N1-mpU
#MRNA40/41/42	mRNA OVA unmodified or 5moU or N1-mpU
#MRNA26/32/33	mRNA CRE unmodified or 5moU or N1-mpU
#MRNA27/28/29	mRNA Cas13d unmodified or 5moU or N1-mpU

Custom mRNAs are also available now!

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This product is sold in accordance with our general conditions of sale that you can find on our website: <https://ozbiosciences.com/content/3-terms-and-conditions>.