Cas13d mRNA
(mRNA encoding Cas13 protein)

Description

Ready-to-use stabilized Cas13d 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: 3184 nt
Molecular weights: #**MRNA27:** 1030940 g/mol; #**MRNA28:** 1044680 g/mol; #**MRNA29**: 1037810 g/mol

Cas13 mRNAs have been designed to produce high expression level of class 2 type VI-D CRISPR-Cas13d system derived from Ruminococcus flavefaciens XPD3002, a recently discovered RNA-guided RNA endonuclease. 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. Cas13d mRNA #**MRNA27** does not bear any additional nucleotide modifications while #**MRNA28** is modified with 5-methoxyuridine (5moU), and #**MRNA29** is modified with N1-methhyl-pseudouridine (N1-mψ) to reduce innate immune response.

Applications

The clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated genes (Cas) system has been rapidly harnessed to perform various genomic engineering tasks. The known Cas13a–Cas13d effectors are able to efficiently cleave complementary target single-stranded RNAs, which represent a potentially safer alternative to deoxyribonuclease Cas9, because it induces loss-of-function phenotypes without genomic loss of the targeted gene. There are currently four subtypes identified in the Cas13 family, including Cas13a (aka C2c2), Cas13b, Cas13c, and Cas13d. All Cas13 family members are smaller than Cas9, with Cas13d being the smallest protein. The Cas13d MRNAs encode for the RNA-guided Cas13d endonuclease used to induce site-directed RNA degradation. Cas13d employs CRISPR-associated RNAs (crRNAs) that contain a customizable 22-nt spacer sequence that can direct the Cas13d protein to specific RNA molecules for targeted RNA degradation. The high catalytic activity of Cas13d in human cells provides a potential mechanism for targeting specific viral RNA genome degradation and viral gene expression inhibition[1,2,3].

1. Granados-Riveron JT., *et al.*, Cancer Res., 2018. DOI: 10.1158/0008-5472.CAN-18-0785.
2. Huynh N., *et al.*, Genome Biol., 2020. DOI:10.1186/s13059-020-02193-y.
3. Abott TR., *et al*., Cell, 2020. DOI:10.1016/j.cell.2020.04.020.

General considerations on OZB's mRNA

Cas13d 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 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 fragment analyzer	✓	✓
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	✓	✓
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.

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, …)

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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

Cas13d mRNAs-20: 20 µg of mRNA.
Cas13d mRNAs-100: 100 µg mRNA.
Cas13d 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
#**MRNA25/30/31**	mRNA Cas9 unmodified or 5moU or N1-mpU
#**MRNA26/32/33**	mRNA Cre unmodified or 5moU or N1-mpU

Custom mRNAs are also available now!

Purchaser Notification | Conditions of Sale

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.