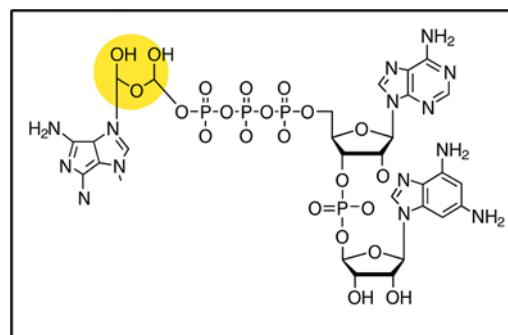


## 1. Product Information

<b>Product name</b>	Cas9 mRNA (Cap1-UNA)
<b>Internal product code</b>	BMr02r
<b>Catalog numbers (aliquot sizes)</b>	560-xxx-GMP (variable sizes)
<b>Quality grade</b>	<b>current Good Manufacturing Practice (cGMP)</b>
<b>Description</b>	mRNA coding for wild type Cas9 nuclease from <i>Streptococcus pyogenes</i> with nuclear localization sequence (NLS-spCas9-NLS), human codon optimization
<b>Length</b>	4417 nt
<b>5'-Cap</b>	Cap1-UNA (m7G(UNA)(5')ppp(5')(2'OMeA)pG)
<b>3'-Poly(A) tail</b>	45 nt
<b>Base modification</b>	N1-methylpseudouridine (m1Ψ)
<b>Concentration</b>	1 mg/ml solution in buffer (frozen)
<b>Storage buffer</b>	1 mM sodium citrate pH 6.4
<b>Storage temperature</b>	-75 ± 15 °C
<b>Lot Nr.</b>	Specified on product label
<b>Use by date</b>	Specified on product label
<b>Manufacturer</b>	Biomay AG, Ada-Lovelace-Straße 2; 1220 Vienna, Austria; <a href="http://www.biomay.com">www.biomay.com</a> ; <a href="mailto:info@biomay.com">info@biomay.com</a>

## 2. Description

Human codon-optimized Cas9 mRNA is translated to a CRISPR nuclease Cas9 (spCas9 from *Streptococcus pyogenes*, uniprot Q99ZW2 / CAS9\_STRP1) with nuclear localization sequences (NLS) on the N- and C-termini. The mRNA is capped with CAP4011 cap analog (Areterna; molecular formula: C<sub>32</sub>H<sub>45</sub>N<sub>15</sub>O<sub>24</sub>P<sub>4</sub>) providing Cap0 (N7-methyl guanosine analog with an unlocked nucleic acid (UNA) connected to the 5' nucleotide through a 5' to 5' triphosphate linkage) and Cap1 (2'-O-methylation at the first position following the Cap0 analog) structures. The product has been manufactured by in vitro transcription using a linear DNA template, purified by chromatography, filtered (0.2μm) and filled as a low-bioburden product.



Cap1-UNA structure of Cas9 mRNA

## 3. Intended Use / Application

Product is mRNA that has been manufactured and quality-controlled under the conditions of current Good Manufacturing Practice (GMP). It has been certified and released by a Qualified Person (QP) under EMA law (EMA directive 2001/83/EC). It was designed and is intended to be used for gene-editing of eukaryotic cells with a specific guide RNA (gRNA).

**Note: Biomay places no restrictions on the use of its Cas9 mRNA products. Depending on the application, end users may need to secure appropriate third-party licenses related to CRISPR/Cas-mediated gene editing or the use of modified nucleosides from the relevant intellectual property holders.**

#### 4. Quality Control and Specifications

	Method	Specification	Results
Appearance	Visual inspection	Clear and colorless solution	Clear and colorless solution
pH-value	pH potentiometric Ph. Eur. 2.2.3	6.4 ± 0.5	6.5
mRNA identity	Denaturing RNA agarose gel electrophoresis	Conforms to reference	Conforms to reference
		Single distinct band visible	Single distinct band visible
	Capillary electrophoresis	Size ± 15 % compared to a reference	Conforms
Sequence identity	Sanger sequencing after reverse transcription	Conforms to theoretical sequence	Conforms
mRNA concentration	UV spectrophotometry (UV absorbance A <sub>260nm</sub> / Ph. Eur. 2.2.25)	0.8 – 1.2 mg/ml	1.1 mg/ml
mRNA integrity	Capillary electrophoresis	≥ 85%	90 %
mRNA purity	UV spectrophotometric (UV absorbance A <sub>260nm</sub> /A <sub>280nm</sub> )	1.8 – 2.3	1.9
5'- Capping efficiency	LC/MS	Report value	95 %
3'- Poly(A)-tail length	LC/MS	Conforms	Conforms
Residual protein	Nano Orange assay	< 1.0 % w/w	< 0.2 % w/w
Residual template DNA	qPCR	< 0.01 % w/w	< 0.0001 % w/w
Endotoxin	LAL test Ph. Eur. 2.6.14 method D	< 5 EU/mg RNA	< 1 EU/mg RNA
Bioburden	Membrane filtration Ph. Eur. 2.6.12	< 1 cfu/ml	< 1 cfu/ml

#### 5. Safety information

Material is considered non-infectious, non-toxic and non-pathogenic under the conditions of the intended applications. General safety procedures should still be followed to maintain a safe working environment. Always wear appropriate personal protective equipment (PPE), including lab coats, gloves, and safety glasses, to avoid contamination and accidental exposure. Work in a clean, organized space, and handle reagents with care, avoiding direct contact. Dispose of all waste materials, including gloves and pipette tips, in designated biohazard containers, even though the commodity is non-hazardous, to prevent cross-contamination. Always wash hands after handling any biological material and before leaving the lab.