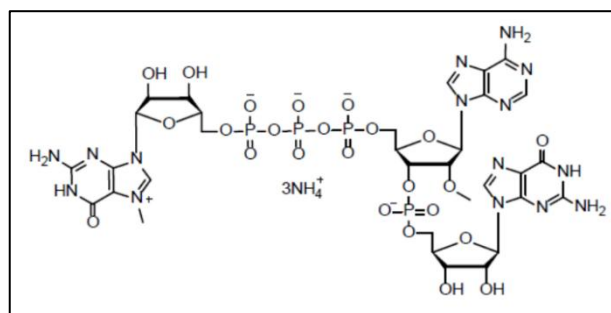


## 1. Product Information

Product name	Cas9 mRNA (Cap1)
Internal product code	BMr08r
Catalog numbers (aliquot sizes)	561-xxx-GMP (variable sizes)
Quality grade	current Good Manufacturing Practice (cGMP)
Description	mRNA coding for wild type Cas9 nuclease from <i>Streptococcus pyogenes</i> with nuclear localization sequence (NLS-spCas9-NLS), human codon optimization
Length	4492 nt
5'-Cap	Cap1 (m7G(5')ppp(5')(2'OMeA)pG)
3' Poly(A) tail	120 nt
Base modification	N1-methylpseudouridine (m1Ψ)
Concentration	1 mg/ml solution in buffer (frozen)
Storage buffer	1 mM sodium citrate pH 6.4
Storage temperature	-75 ± 15 °C
Lot Nr.	Specified on product label
Use by date	Specified on product label
Manufacturer	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 CAP3011 cap analog (Areterna; molecular formula: C<sub>32</sub>H<sub>43</sub>N<sub>15</sub>O<sub>24</sub>P<sub>4</sub>) providing Cap0 (N7-methyl guanosine analog connected to the 5' nucleotide through a 5' to 5' triphosphate linkage analog) 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 (0.2 µm) and filled as a low-bioburden product.



Cap1 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	$6.4 \pm 0.5$	6.4
mRNA identity	Denaturing RNA agarose gel electrophoresis	Conforms to reference	Conforms to reference
		Single distinct band visible	Single distinct band visible
	Capillary electrophoresis	Size $\pm 15\%$ of theoretical size	Conforms
Sequence identity	Sanger sequencing after reverse transcription	Conforms to theoretical sequence	Conforms
mRNA concentration	UV spectrophotometry (UV absorbance $A_{260nm}$ / Ph. Eur. 2.2.25)	0.8 – 1.2 mg/ml	1.04 mg/ml
mRNA integrity	Capillary electrophoresis	$\geq 80\%$	94 %
mRNA purity	UV spectrophotometric (UV absorbance $A_{260nm}/A_{280nm}$ )	1.8 – 2.3	1.9
5'- Capping efficiency	LC/MS	$\geq 80\%$	99 %
3'- Poly(A)-tail length	LC/MS	$\geq 90\%$ (of theoretical poly(A) tail length)	Conforms
Residual protein	Nano Orange assay	$< 1.0 \%$ w/w	$< 0.3 \%$ w/w
Residual template DNA	qPCR	$< 1$ ng/mg RNA	0.016 ng/mg RNA
dsRNA	ELISA	$\leq 0.150 \%$	0.068 %
Endotoxin	LAL test Ph. Eur. 2.6.14 method D	$\leq 1.0$ EU/mg RNA	$< 0.1$ 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.