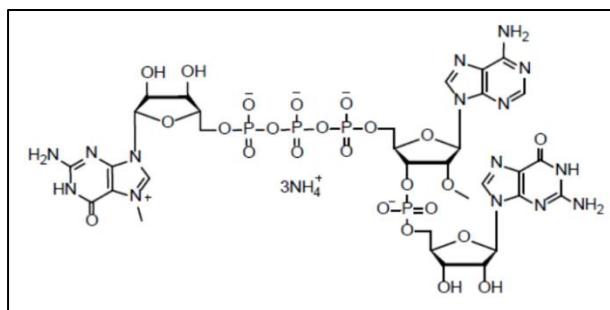


## 1. Product Information

<b>Product name</b>	Cas9 mRNA (Cap1)
<b>Internal product code</b>	BMr08r
<b>Catalog numbers (aliquot sizes)</b>	161-0025-RUO (25 µg) 161-0100-RUO (100 µg) 161-1000-RUO (1000 µg)
<b>Quality grade</b>	<b>Research use only (RUO)</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>	4492 nt
<b>5'-Cap</b>	Cap1 (m7G(5')ppp(5')(2'OMeA)pG)
<b>3' Poly(A) tail</b>	120 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 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 (0.2µm) and filled as a low-bioburden product.



Cap1 structure of Cas9 mRNA

## 3. Intended Use / Application

**Research use only:** product is an mRNA manufactured and tested under laboratory conditions. It was designed and is intended to be used for gene-editing of eukaryotic cells with a specific guide RNA (gRNA). Example applications are CRISPR/Cas mediated knockout / knock-in in cell culture or animal models for research and development. The material may not be used for administration to humans, or in human clinical trials (Phases I, II

or III), nor for any commercial applications, neither directly (as mRNA) nor indirectly (Cas9 gene-edited products from cell culture). **Material is intended for research and development use only.**

**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.**

## Quality Control and Specifications

Parameter	Method	Specification	Results
Appearance	Visual inspection	Clear and colorless solution	Clear and colorless solution
pH-value	pH potentiometric Ph. Eur. 2.2.3	$6.4 \pm 0.5$	6.5
mRNA identity	Denaturing RNA agarose gel electrophoresis	Conforms to reference	Conforms to reference
		Single distinct band visible	Single distinct band visible
	Capillary electrophoresis	Size $\pm 15\%$ compared to a reference	Conforms
Sequence identity	Sanger sequencing after reverse transcription	Conforms to theoretical sequence	Conforms
mRNA concentration	UV spectrophotometry (UV absorbance $A_{260\text{nm}}$ / Ph. Eur. 2.2.25)	0.8 – 1.2 mg/ml	1.1 mg/ml
mRNA integrity	Capillary electrophoresis	$\geq 80\%$	94%
mRNA purity	UV spectrophotometric (UV absorbance $A_{260\text{nm}}/A_{280\text{nm}}$ )	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

## 4. 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. **The material may not be administered to humans or used human clinical trials.** The material may be used for laboratory research only.