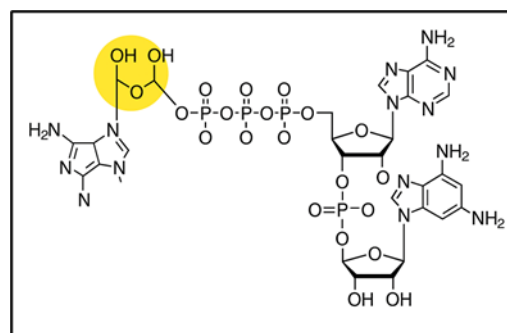


1. Product Information

Product name	Cas9 mRNA (Cap4)
Internal product code	BMr02r
Catalog numbers (aliquot sizes)	160-0025-RUO (25 µg) 160-0100-RUO (100 µg) 160-1000-RUO (1000 µg)
Quality grade	Research use only (RUO)
Description	mRNA coding for wild type Cas9 nuclease from <i>Streptococcus pyogenes</i> with nuclear localization sequence (NLS-spCas9-NLS), human codon optimization
Length	4417 nt
5'-Cap	Cap4 (CAP4011™, Areterna)
3' Poly(A) tail	45 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; www.biomay.com ; info@biomay.com

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: C₃₂H₄₅N₁₅O₂₄P₄) providing Cap-0 (N7-methyl guanosine analog connected to the 5' nucleotide through a 5' to 5' triphosphate linkage) and Cap-1 (2'-O-methylation at the first position following the Cap-0 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.



Cap-1 structure of Cas9 mRNA: CAP4011 from Areterna (www.areterna.com)

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

	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 $\pm 10\%$ compared to a reference	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	1.1mg/ml
mRNA homogeneity	Capillary electrophoresis	Homogeneity $\geq 85\%$	90%
mRNA purity	UV spectrophotometric (UV absorbance A_{260nm}/A_{280nm})	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	$< 1.0 \% \text{ w/w}$	$< 0.2 \% \text{ w/w}$
Residual template DNA	qPCR	$< 0.01 \% \text{ w/w}$	$< 0,0001 \% \text{ w/w}$
Endotoxin	LAL test Ph. Eur. 2.6.14 method D	$< 5 \text{ EU/mg RNA}$	$< 1 \text{ EU/mg RNA}$
Bioburden*)	Membrane filtration Ph. Eur. 2.6.12	$< 1 \text{ cfu/ml}$	$< 1 \text{ cfu/ml}$

*) Note: product is a 0.2 μm filtrated low-bioburden solution. It is not aseptically filled (not sterile).

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.