



mRNA Cap 2'-O-Methyltransferase, GMP Grade

Cat. No.: GMP-M072 animal-free, ampicillin-free

01/ Product Description

With S-adenosylmethionine (SAM) as the methyl group base donor, mRNA Cap 2'-O-Methyltransferase can add a methyl group to the 2'-O of the first nucleotide of the Cap0 structure, [$m^7G\text{pppN}_1(\text{pN})x\text{-OH}(3')$], next to the RNA 5' end. Methylation of capped mRNA from Cap 0 to Cap1 naturally exists in eukaryocyte, where the Cap1 structure shows an important effect for translation, thus for expression as well. This product doesn't show a reactivity to RNA substrate other than RNA with Cap0 structure.

This product is from a large scale GMP leveled recombinant Cap 2'-O-Methyltransferase production via *E. coli* expression. Applying pharmaceutical leveled adjuvant and material for production, strictly controlling host protein residues, nucleic acid residues and other impurities, we guarantee manufacture and quality control practice complying to GMP regulation, as well as all the materials traceable.

This product has completed the DMF record of FDA and passed the HALAL certification.

Chemical structure of the RNA cap

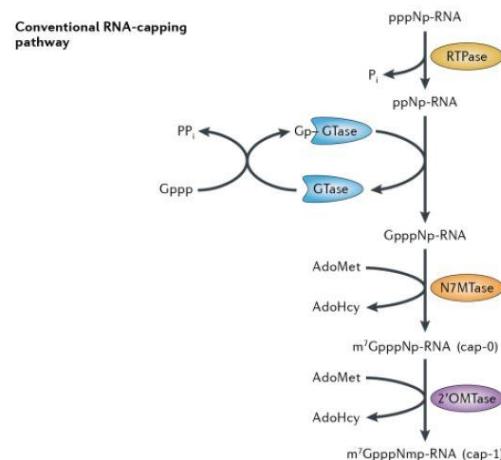
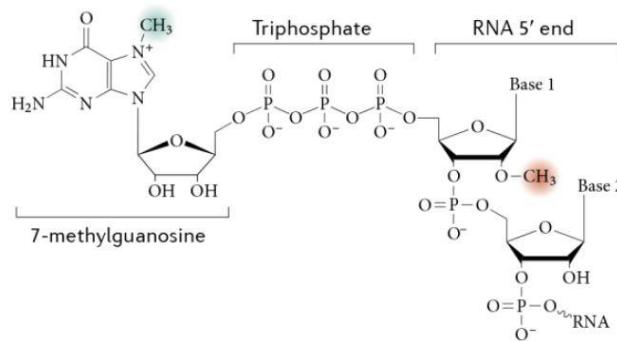


Figure 1. mRNA cap structure 2'-O-methyl transferase mechanism of action. The cap structure of mRNA is composed of a 7-methylguanosine connected to the 5' nucleoside of the mRNA chain through a 5'-5' triphosphate bridge. The Cap-0 structure is formed by the sequential reaction of three enzymes between adjacent RNA strands. Further formation of cap-1 structure requires the participation of 2'-O-methyltransferase, and this modification can reduce the cellular innate immune response caused by RNA in vivo. This figure is quoted from Decroly, E., Ferron, F., Lescar, J. et al. (2012). Conventional and unconventional mechanisms for capping viral mRNA. *Nat Rev Microbiol* 10, 51–65.

02/ Reaction Principle

Capping process is composed of 4 steps, with 4 reactions.

- 1) $\text{pppN}_1(\text{pN})x\text{-OH}(3') \rightarrow \text{ppN}_1(\text{pN})x\text{-OH}(3') + \text{Pi}$
- 2) $\text{ppN}_1(\text{pN})x\text{-OH}(3') + \text{GTP} \rightarrow \text{G}(5')\text{ppp}(5')\text{N}_1(\text{pN})x\text{-OH}(3') + \text{PPi}$
- 3) $\text{G}(5')\text{ppp}(5')\text{N}_1(\text{pN})x\text{-OH}(3') + \text{AdoMet} \rightarrow \text{m}7\text{G}(5')\text{ppp}(5')\text{N}_1(\text{pN})x\text{-OH}(3') + \text{AdoHyc}$
- 4) $\text{m}7\text{GpppN}_1(\text{pN})x\text{-OH}(3') + \text{AdoMet} \rightarrow \text{m}7\text{Gppp}[\text{m}^2\text{-O}]\text{N}_1(\text{pN})x\text{-OH}(3') + \text{AdoHyc}$

This process grants mRNA more stable and more efficient in translation. With 2'-O-methyltransferase (GMP-M072, Novoprotein), we may turn the “Cap0” into “Cap1”, which better improves efficiency of translation, decreasing immunogenicity at the same time.

A standard Vaccinia Capping System reaction can cap about 10 μg of RNA ($\geq 100\text{nt}$), with proportional amplification and diminution realizable. We can add poly(A) with (GMP-M012, Novoprotein) at the 3' end of RNA.

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03/ Quality Criterion

Element	Standard
Appearance	Clear and transparent solution
Identification	Positive
Visible Particles	Meet the specification
pH	7.5-8.5
Activity	50KU/ml-52.5KU/ml
Purity	≥95%
Protein Content	Meet the specification
Endonuclease residues	The degradation of substrate was ≤10%
Exonuclease residues	The degradation of substrate was ≤10%
RNase Residues	The degradation of substrate was ≤10%
Bacterial Endotoxins	<5EU/ml
Exogenous DNA Residues	≤100pg/mg
Host-cell Protein Residues	≤50ppm
Mycoplasma	Negative
Heavy Metals	≤10ppm
Microbial Limit	Total aerobic microbial count≤1cfu/10ml, total yeasts and molds count≤1cfu/10ml

04/ Complying to Following Regulations

1. ISO 9001:2015, certified facility.
2. GMP Appendix – Cellular therapeutic product National Medical Products Administration.
3. The Pandect of Genetic Therapeutic Product for Human Chinese Pharmacopoeia Commission.
4. USP Chapter <1043>, Ancillary Materials for Cell, Gene, and Tissue-Engineered Products.
5. USP Chapter <92>, Growth Factors and Cytokines Used in Cell Therapy Manufacturing.
6. Ph. Eur. General Chapter 5.2.12, Raw Materials of Biological Origin for the Production of Cell-based and Gene Therapy Medicinal Products.

05/ Application

Methylate the 2'-O of the first nucleotide of the Cap0 structure, to realize mRNA translation and expression.

06/ Essential Product Information

Source	Purified from genetically modified <i>E. coli</i> expression, carrying Cap 2'-O-methyltransferase gene
Reaction Condition	1×Capping Buffer (50mM Tris-HCl, pH 8.0; 5mM KCl, 1mM MgCl ₂ , 1mM DTT). Incubation 37°C
Storage Buffer	20mM Tris-HCl pH8.0; 100mM NaCl; 1mM DTT; 0.1mM EDTA; 0.1% TritonX-100; 50% (v/v) Glycerol
Storage Conditions	At -20±5°C
Unit Definition	At 37°C, within 1 hour, the amount of enzyme required that will methylate 10pmol Cap0-RNA transcript of 80 nucleotide (80nt) transcript is defined as one unit of enzyme activity.

07/ Product Packaging

SKU	Components	Volume
GMP-M072-01A	mRNA Cap 2'-O-Methyltransferase, GMP Grade (50U/μl)	50μl
GMP-M072-M001	mRNA Cap 2'-O-Methyltransferase, GMP Grade (50U/μl)	1ml
GMP-M072-M010	mRNA Cap 2'-O-Methyltransferase, GMP Grade (50U/μl)	10ml
GMP-M072-M050	mRNA Cap 2'-O-Methyltransferase, GMP Grade (50U/μl)	50ml

08/ Precautions

1. **SAM** is unstable at pH 7–8, 37 °C and needs to be freshly prepared before the reaction starts. The amount of SAM can be calculated in advance, and the aliquoted 32 mM stock solution is diluted into a 2mM working solution before the reaction starts. To avoid SAM degradation, the working solution needs to be kept on ice.
2. Prepare **Cap0-capped RNA** for Cap0 to Cap1 methylation. Before starting reaction, the RNA applied for capping should be purified and dissolved in nuclease-free water before. The solution for use must NOT contain any EDTA or salt. We may obtain Cap0-capped RNA by Vaccinia Capping System (GMP-M062, Novoprotein). We may run both Cap0 capping and Cap1 capping in one reactor at the same time. Equally, we may realize cap1 capping conveniently by applying capping reaction kit (M082, Novoprotein).
3. RNA transcript with **5' end secondary structure** can be an obstacle for capping. Heating the RNA sample can remove complex structure of the transcript. We may extend heating time to 10 minutes, and extend the capping reaction time to 60 minutes.
4. When used for the capped RNA 3'-poly (A) tailing, we may apply *E. coli* Poly(A) Polymerase, GMP Grade (GMP-M012, Novoprotein).
5. The sample needs to be purified before transfection.
6. When adding 0.5 μl of **RNase inhibitor** (GMP-E125, Novoprotein) to the reaction system (shown below in “Manual” part), please deduct equal volume of RNase-free water.
7. Do **NOT** store the product at -70°C or an even lower temperature.
8. In case of **white precipitation in reaction buffer** (shown below in “Manual” part), incubate it at 37 ° C for 5 mins, blend it, totally dissolving precipitation.

09/ Protocol

1. Capping Reaction (20μl)

This manual guides for the typical capping reaction of 10μg RNA (\geq 100 nt). It is possible to scaled up to a greater level for further research purpose.

- 1) Dilute an appropriate amount of RNA to 16μl with RNase Free Water.
- 2) The RNA was heated at 65°C for 5min to denature, and then placed on ice for 5min.
- 3) Dilute 32mM SAM to 2mM.
- 4) Add the components shown below in the following form according to the order up to down:

Components	Volume
Denatured Cap0-capped RNA	16μl
10x Capping Reaction Buffer, GMP Grade	2μl
SAM, GMP Grade (4mM)	1μl
mRNA Cap 2'-O-methyltransferase, GMP Grade (50U/μl)	1μl

- 5) Incubate at 37 ° C for 60 mins; For RNAs less than 200nt in length, the incubation time was extended to 2 hours.

10/ FAQs

1. low capping efficiency

1) Before capping reaction, RNA should be purified to remove residual proteins, contaminants and unbound nucleotides, and dissolved in RNase-free water, not in EDTA or other salt solutions.

2) SAM will degrade slowly at room temperature and should always be placed on ice. The degradation of SAM will lead to low N7 methylation efficiency, which will further lead to capping failure.

3) The conditions of RNA thermal denaturation can be appropriately adjusted, and the heating time can be extended to 10min, and the reaction time with capping can be extended to 60min;

4) Some RNA forms stable structures (such as source dimers, hairpin structures) at the 5' terminal, restricting the access of capping enzymes. RNA denaturation temperature can be increased after sequence analysis. If the 5' terminal is highly structured, the sequence needs to be modified by molecular biology techniques. This is usually achieved by a single point mutation in the first five bases of a DNA template that transcribes RNA (non-coding regions).

2. White precipitate occurs in the buffer.

1) The reaction buffer was incubated at 37°C for 5min and thoroughly mixed to dissolve the precipitate;

2) Do not store the kit at -70°C.

11/ Related Product

Cat. No.	Product Name	Cat. No.	Product Name
GMP-M062	Vaccinia Capping Enzyme, GMP Grade	GMP-E125	RNase Inhibitor, GMP Grade
GMP-M036	Pyrophosphatase, Inorganic (yeast), GMP Grade	GMP-E127	DNase I, GMP Grade
GMP-M012	<i>E. coli</i> Poly(A) Polymerase, GMP Grade	GMP-E121	T7 RNA Polymerase, GMP Grade
GMP-EB62	10x Capping Reaction Buffer, GMP Grade	GMP-S062	SAM, GMP Grade
GMP-S024N	GTP, GMP Grade		