Tinzyme Co., Limited

Email: sales@tinzyme.com Website: www.tinzyme.com
Tel: +86-755-86134126 WhatsApp/Facebook/Twitter: +86-189-22896756

mRNA Cap 2´ O Methyltransferase, GMP Grade

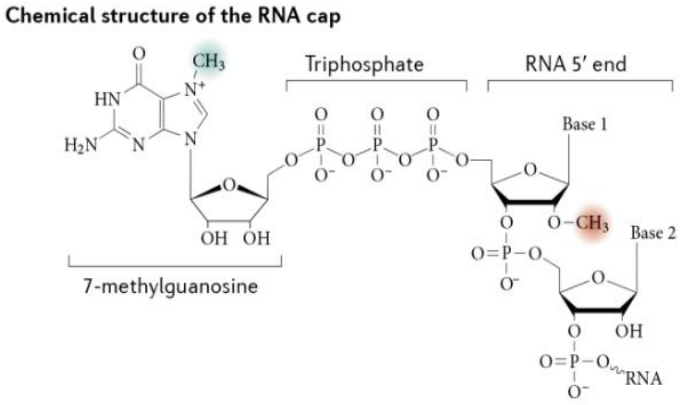
Product Number: GMP-M072 Animal-free Ampicillin-free

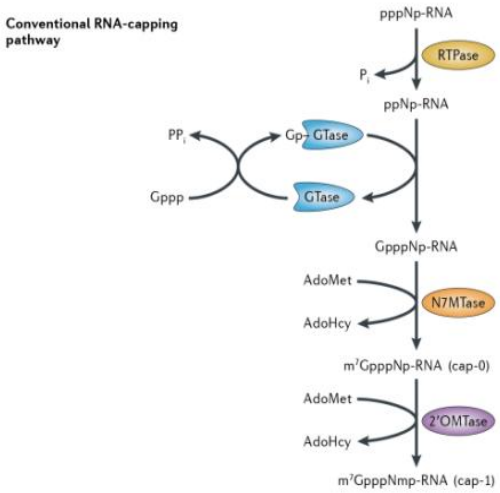
Description

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, [m7GpppN1(pN)x-OH(3')], next to the RNA 5´ end. Methylation of capped mRNAfrom 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.





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.

Source	Purified from genetically modified E. coli expression, carrying Cap 2'-O-methyltransferase gene
Reaction conditions	1×Capping Buffer (50 mM Tris-HCl, pH 8.0; 5 mM KCl, 1 mM MgCl2, 1 mM 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;
Activity 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.

The Principle of Capping

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

1. $pppN_1(pN)_x$-OH(3') → $ppN_1(pN)_x$-OH(3') + Pi
2. $pppN_1(pN)_x$-OH(3') + GTP → G(5')ppp(5')$N_1(pN)_x$-OH(3') + PPi
3. G(5')ppp(5')$N_1(pN)_x$-OH(3') + AdoMet → m7G(5')ppp(5')$N_1(pN)_x$-OH(3') + AdoHyc

4. $m^7GpppN_1(pN)_x$-OH(3') + AdoMet $m^7Gppp[m^{2'-O}]N_1(pN)_x$-OH(3') + AdoHyc

This process grants mRNA more stable and more efficient in translation. With mRNA Cap 2′ O Methyltransferase, GMP Grade (GMP-M072, Novoprotein), we may turn the "Cap0" into "Cap!", which better improves efficiency of translation, decreasing immunogenicity at the same time.

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

Quality Elements

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%
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 Metal Residues	≤10ppm

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.

Application

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

Package

Components	Volume
mRNA Cap 2'-O-Methyltransferase, GMP Grade (50U/μl)	50μl
mRNA Cap 2'-O-Methyltransferase, GMP Grade (50U/μl)	1ml
mRNA Cap 2'-O-Methyltransferase, GMP Grade (50U/μl)	10ml
mRNA Cap 2'-O-Methyltransferase, GMP Grade (50U/μl)	50ml

Note

1. SAM is unstable at pH7–8, 37°C and needs to be freshly prepared before the reaction starts. The amount of SAM can be calculated in advance, and the aliquoted 32mM 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 Enzyme, GMP Grade (GMP-M062, Novoprotein). We may run both Cap0 capping and Cap1 capping in one reactor at the same time.
3. RNA transcript with 5' end in complex can be an obstacle for capping. In case 5' end in complex, 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 60mins.
4. When used for the capped RNA 3'-poly (A) tailing, we may apply Poly(A) Polymerase, GMP Grade (GMP-M012, Novoprotein).
5. The sample needs to be purified before transfection.
6. When adding 0.5μl of RNase Inhibitor, GMP Grade (GMP-RI01, 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 a 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.

Protocol

1. Capping Reaction (20μl)

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

1.1. Dilute an appropriate amount of RNA to 16μl with RNase Free Water.

1.2. The RNA was heated at 65°C for 5min to denature, and then placed on ice for 5min.

1.3. Dilute 32mM SAM to 2mM.

1.4. Add the components shown below in the following form according to the order up to down:

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

1.5. Incubate at 37°C for 60mins; In case RNA with a length smaller than 200nt, incubate at 37°C for 2hs.

FAQs

1. low capping efficiency

1.1. 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.

1.2. 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.

1.3. 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;

1.4. 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.

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

2.2. Do not store the kit at -70℃.

Related Products

Product Number	Product Name
GMP-M062	Vaccinia Capping Enzyme, GMP Grade
GMP-RI01	RNase Inhibitor, GMP Grade
GMP-M012	Poly(A) Polymerase, GMP Grade
GMP-DI05	DNase I Recombinant GMP grade
GMP-T701	T7 RNA Polymerase, GMP Grade
GMP-M036	Pyrophosphatase, Inorganic (yeast), GMP Grade (ppase)
GMP-E131	T7 High Yield RNA Transcription kit, GMP Grade
GMP-SAM01	SAM 32mM solution
GMP-EB62	10×Capping Buffer
D1331	dATP 100mM solution
D2331	dGTP 100mM solution
D3331	dCTP 100mM solution
D4331	dTTP 100mM solution
N5331	N1-Me-Pseudo UTP,100mM Solution