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Vaccinia Capping Enzyme, GMP Grade

Product Number: GMP-M062 Animal-free Ampicillin-free

Description

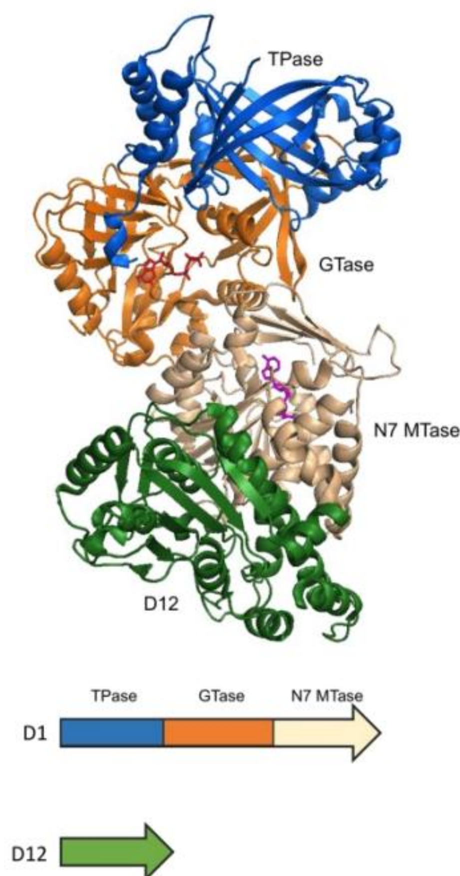
The vaccinia capping enzyme together with substrates, is capable to add the 7-methylguanosine capping structure (m7Gppp, Cap0) to the 5´ end of the RNA. In eukaryotes, this structure is closely related to the stability, transport, and translation of mRNA. Capping RNA with an enzymatic reaction is a simple and effective method that can significantly improve the stability and translation capabilities of RNA used for in vitro transcription, transfection, and micro-injection. The vaccinia virus capping enzyme consists of two subunits (D1 and D12) (Figure 1a), and possesses RNA triphosphatase, guanosine transferase, and guanine methyltransferase activities, and they are all necessary for adding a complete Cap0 structure m7Gppp5´N to mRNA (Figure 1b).

This product can be applied to capping reaction of RNA generated by theT7 RNA Polymerase, GMP Grade（GMP-T701，Novoprotein）The capping reaction is completed within an hour and the efficiency is close to 100% with correct direction.

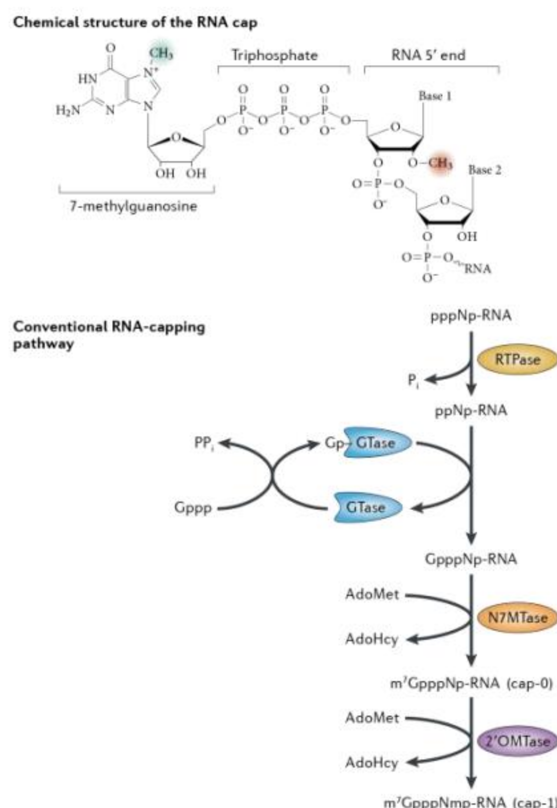
Vaccinia Capping Enzyme is GMP Grade produced in E. coli. Our manufacturing processes are strictly controlled to ensure the end products free from host protein or nucleic acid contaminations and other impurities following the Pharmaceutical Manufacturing Guidelines. We guarantee the manufacturing and quality control comply with GMP regulation for tracking each and every step of the manufacturing process, including raw material sourcing.

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

a.



b.



The structure and mechanism of vaccinia virus capping.

a. Co-crystal structure of vaccinia virus capping enzyme and GTP (red), SAH (rose red) (PDB 4CKB). The enzyme consists of two subunits, D1 and D12, and has the functions of RNA triphosphatase (blue), guanosine transferase (orange) and guanine methyltransferase activities (beige). This figure is quoted from Ramanathan, A., Robb, GB, & Chan, SH (2016). mRNA capping:biological functions and applications. Nucleic Acids Res 44 (16), 7511–7526.

b. The cap structure of mRNA is composed of a 7-Methylguanosine consists of a 5’-5’ triphosphate bridge connected to the 5’ nucleoside of the mRNA chain. 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. 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 the vaccinia capping enzyme gene
Reaction Condition	1×Capping Buffer (50mM Tris-HCl, pH 8.0; 5mM KCl, 1mM $MgCl_2$, 1mM DTT). Requiring37°C incubation
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 incorporate 10pmol (α-32P) GTP into an 80 nucleotide (80nt) transcript is defined as one unit of enzyme activity

Reaction Principle

Capping process is composed of 3 steps, with 3 reactions.

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

This process is called “Capping". It grants mRNA more stable and more efficient in translation. The capped structure is called “Cap0".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 10μg of RNA (≥100nt), with proportional amplification and diminution realizable. We can add Poly(A) Polymerase, GMP Grade with (GMP-M012, Novoprotein) at the 3' end of RNA.

Quality Criterion

Element	Standard
Appearance	clear and transparent solution
Identification	Positive
Visible impurities	Meet the specification
pH	7.5-8.5
Active	10kU/ml-10.5kU/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

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

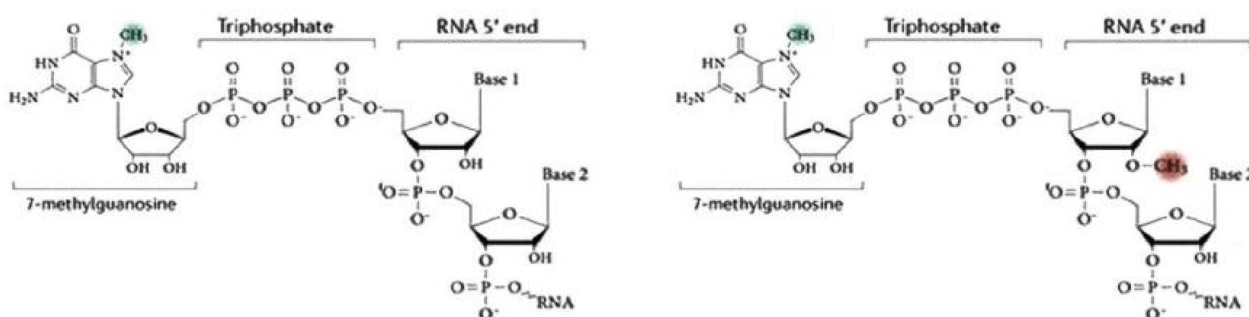
Adding the 7-methylguanosine capping structure (m7Gppp, Cap0) and relative decoration mark to the 5´end of the mRNA.

Package

Components	Volume
Vaccinia Capping Enzyme, GMP Grade (10U/μl)	50μl
Vaccinia Capping Enzyme, GMP Grade (10U/μl)	1ml
Vaccinia Capping Enzyme, GMP Grade (10U/μl)	10ml
Vaccinia Capping Enzyme, GMP Grade (10U/μl)	50ml

Note

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 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. Before starting reaction, the RNA applied for capping should be purified and dissolved in nuclease-free water. The solution for use must NOT contain any EDTA or salt.
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. Capping from Cap0 to Cap1 naturally exists in eukaryotic cells, which enhance translation efficiency.



Vaccinia Capping Enzyme, GMP Grade(GMP-M062) can co-work with mRNA Cap2´O Methyltransferase, GMP Grade (GMP-M072). When using new cell lines or translation systems, it is recommended to compare the translation efficiency and immunogenicity of Cap 0 and Cap 1-mRNA to determine the optimal Cap structure.

5. 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.
6. When used for RNA 5´ -end labeling, GTP should be diluted to 1-3 times of the mol concentration of mRNA.
7. When used for the capped RNA 3'-poly (A) tailing, we may apply Poly(A) Polymerase, GMP Grade (GMP-M012, Novoprotein).
8. The sample needs to be purified before transfection.
9. **Do NOT** store the product at **-70°C** or an even lower temperature.
10. 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 (≥100nt). It is possible to scaled up to a greater level for further research purpose.

1.1. Dilute an appropriate amount of RNA to 15μl with RNase Free Water.
1.2. The RNA was heated at 65°C for 5min to denature, and quickly 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	20μl
Denatured RNA	15μl
10× Capping Reaction Buffer, GMP Grade	2μl
GTP, GMP Grade (10mM)	1μl
SAM, GMP Grade (2mM)	1μl
Vaccinia Capping Enzyme, GMP Grade (10U/μl)	1μl

1.5. Incubate at 37°C for 30 min.

2. 5′ -end Labeling Reaction (20μl)

This manual guides for the typical RNA labeling with triphosphate at the 5'end. It is possible to scaled up to a greater level for further research purpose. The labeling efficiency is affected by the molar ratio of RNA / GTP in the reaction system and the GTP content in the RNA sample.

2.1. Dilute an appropriate amount of RNA to 14μl with RNase Free Water.
2.2. The RNA was heated at 65°C for 5min, and then placed on ice for 5min .
2.3. Dilute 32mM SAM to 2mM.
2.4. Add the components shown below in the following form according to the order up to down:

Components	20μl
Denatured RNA	14μl
10× Capping Reaction Buffer, GMP Grade	2μl
GTP mix*	2μl
SAM, GMP Grade (2mM)	1μl
Vaccinia Capping Enzyme, GMP Grade (10U/μl)	1μl

2.5. Incubate at 37°C for 30 min.

Annotation: GTP mix consists of GTP and a small amount of markers, GTP solution should be diluted to 1-3 times of the mol concentration of mRNA in the reaction system.

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℃ 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-M072	mRNA Cap 2´ O Methyltransferase, 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-EB62	10X Capping Buffer
GMP-SAM01	SAM 32mM solution
GMP-E131	T7 High Yield RNA Transcription kit, GMP Grade
D1331	dATP 100mM solution
D2331	dGTP 100mM solution
D3331	dCTP 100mM solution
D4331	dTTP 100mM solution
N5331	N1-Me-Pseudo UTP,100mM Solution