Tinzyme Co., Limited

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Cap 1 Capping System Kit

Product Number: CP082

Shipping and Storage

-20℃.

Components

Component	50rxns	100rxns
Vaccinia Capping Enzyme (10U/μl)	40μl	400μl
mRNA Cap 2´-O-Methyltransferase (100 U/μl)	40μl	400μl
SAM(20 mM)	25μl	250μl
10 mM GTP	100μl	1ml
Recombinant RNase Inhibitor (40 U/μl)	25μl	250μl
10×Capping Reaction Buffer	100μl	1ml
RNase Free Water	1ml×2	1.2ml×6

User provided materials: IVT RNA, RNA purification reagents or magnetic beads

Description

The cowpox virus cap system utilizes cowpox virus cap enzymes and related components to add 7-methylguanosine cap structures (m^7Gppp, Cap0) to the 5 'end of RNA. In eukaryotes, this structure is closely related to the stability, transport, and translation of mRNA. The use of enzymatic reactions to cap RNA is a simple and effective method, and the cap structure is completely consistent with the natural Cap0 structure, which can significantly improve the stability and translation ability of RNA used for in vitro transcription, transfection, and microinjection. This enzyme is composed of two subunits (D1 and D12), which combine the functions of RNA triphosphatase, guanyltransferase, and guanine methyltransferase. They are necessary for adding a complete Cap 0 structure m^7Gppp5'N (Figure 1).

The capped RNA product constructed from cowpox virus cap enzyme has a "cap 0" structure. By simultaneously using mRNA Cap 2'- O-methyltransferase and cowpox virus capping enzyme in the capping reaction, Cap 0-RNA can be transformed into a "cap 1" structure. MRNA Cap 2'- O-methyltransferase prepares Cap 1-RNA from Cap 0-RNA by transferring methyl groups from the donor molecule SAM to the 2' - O position of the first nucleotide adjacent to the cap structure at the 5 'end of cap 0-RNA.

Source	E. coli
Reaction conditions	1×Capping Buffer (50 mM Tris-HCl, pH 8.0; 5 mM KCl, 1 mM MgCl2, 1 mM DTT). Incubate at 37°C
Storage buffer	20mM Tris-HCl pH8.0, 100mM NaCl, 1mM DTT, 0.1mM EDTA, 0.1% TritonX-100, 50% glycerol
Storage method	Store at -20°C, please repack immediately upon receipt to avoid repeated freeze-thaw cycles

Figure 1 Participate in the enzymatic pathway of mRNA capping. The production of Cap 0 structural RNA requires cowpox virus cap enzyme: this enzyme combines the functions of triphosphatase, guanyltransferase, and guanine methyltransferase. Among them, S-adenosylmethionine (SAM) is a methyl donor. Once the Cap 0 structure is generated, it can be further modified by 2 '-O-ribomethyltransferase to produce the Cap 1 structure. This image is taken from Michael Beverly, Amy Dell, Parul Parmar, Leslie Houghton et al. (2016) Label free analysis of mRNA capping efficiency using RNase H probes and LC-MS Anal Bional Chem.

This system can be used for the capping reaction of IVT RNA, allowing the capping reaction to be completed within 1 hour, with an efficiency close to 100%, and ensuring the correct direction. IVT recommends using the T7 High Yield RNA Transcription kit (product code: E131).

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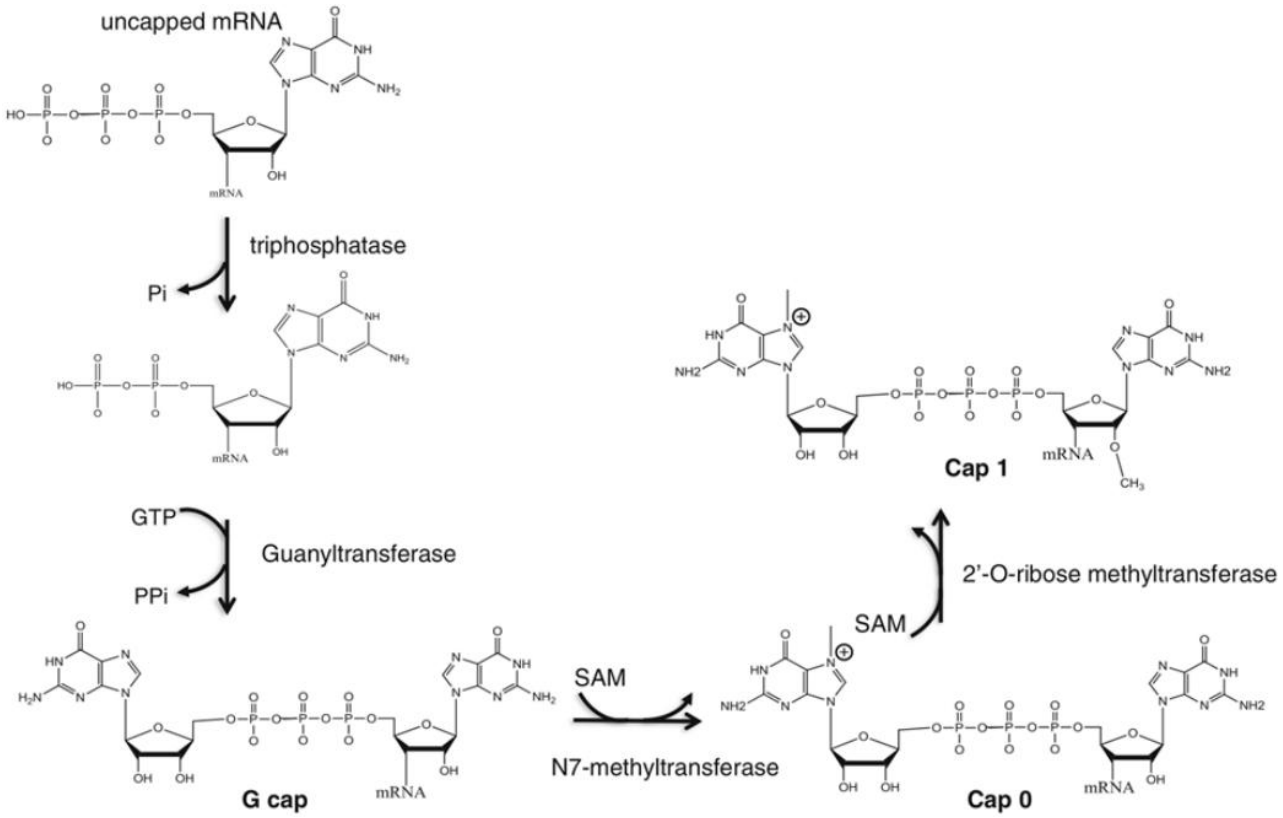

Capping process

The Cap 1 Capping System catalyzes four enzymatic reactions:

1. RNA triphosphatase cleaves RNA 5 '- triphosphate into diphosphate.
   PppN1 (p) Nx OH (3 ') → ppN1 (pN) x-OH (3')+Pi
2. RNA guanylate transferase connects GTP to 5 '- diphosphate of RNA N1.
   PpN1 (pN) x-OH (3 ')+GTP → G (5') ppp (5 ') N1 (pN) x-OH (3')+PPi
3. Guanine 7-methyltransferase, using S-adenosylmethionine as a cofactor, catalyzes the 7-nitromethylation of guanine.
   G (5 ') ppp (5') N1 (pN) x-OH (3 ')+AdoMet → m7G (5') ppp (5 ') N1 (pN) x-OH (3')+AdoHyc
4. MRNA Cap 2 '- O-methyltransferase can add a methyl group to the 2' - O of the first nucleotide adjacent to the cap structure at the 5 'end of Cap 0-RNA.
   M7GpppN1 (pN) x-OH (3 ')+AdoMet m7Gppp [m2' - O] N1 (pN) x-OH (3 ')+AdoHyc

Application

1. Capping of mRNA before in vivo or in vitro translation and labeling of the 5 'end of mRNA.
2. Improve the translation efficiency of RNA;
3. Improve mRNA expression after microinjection and transfection.

Quality control

No residue of Escherichia coli DNA, no residue of nucleic acid endonucleases and exonucleases, and no residue of RNA enzymes.

Note

1. **SAM:** SAM is unstable under pH 7-8 and 37°C. To avoid degradation of SAM, the working solution needs to be stored on ice.
2. **RNA:** Before using the cap system, the RNA produced by the in vitro transcription reaction should be purified and dissolved in RNase free water. RNA should not be dissolved in EDTA solution or other salt solutions.

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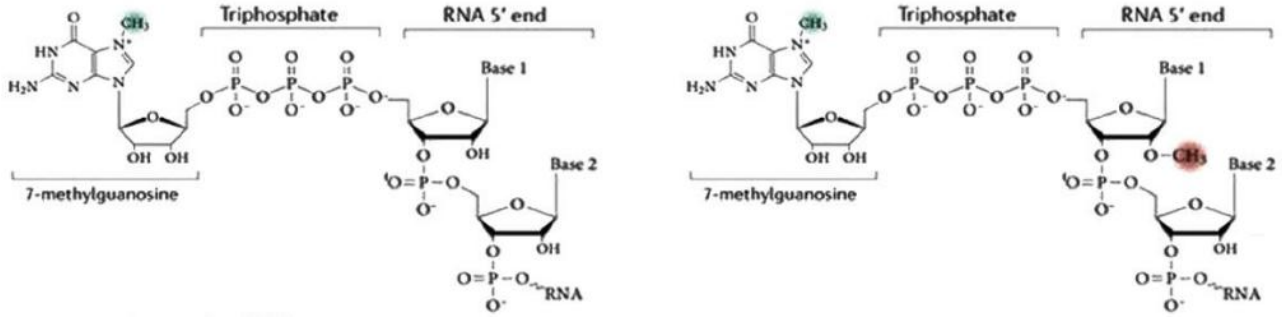
3. **RNA secondary structure:** Some RNA transcripts may form stable secondary structures (homodimers and hairpin structures), and if the secondary structure occurs at the 5 'end of the transcript, it can affect the efficiency of capping. Heating the RNA before reacting with the capping enzyme can remove the secondary structure at the 5 'end of the transcript. If the 5' end structure of the transcript is complex, the heating time can be extended to 10 minutes, and the capping reaction time can be extended to 60 minutes.
4. **Cap 0- and Cap 1-mRNA**:The difference between Cap 0- and Cap 1 mRNA lies in whether the first nucleotide (N1) adjacent to the cap structure at the 5 'end of RNA has a 2' - O-methyl group. In higher eukaryotic cells, this methylation is part of the natural capping process, and the Cap 1 structure enhances the translation efficiency of mRNA.



5. **Poly (A) tail:** If the capped RNA requires the addition of a 3 '- poly (A) tail, Poly (A) Polymerase (product code: M012) can be used for tail addition. The RNA with caps and tails needs to be purified before conducting RNA transfection experiments.
6. **RNase Inhibitor:** When configuring the reaction system, 0.5ul of RNase Inhibitor, GMP Grade (product code: GMP-RI01) can be added, while removing an equal volume of RNase free water.

Protocol

Capping reaction (reaction system 100ul)

This step is applicable to the capping reaction of 50ug RNA (≥ 100 nt) and can be scaled up according to experimental needs.

1. Dilute an appropriate amount of RNA to 67ul using RNase free water;
2. Heat the RNA at 65℃ for 5 minutes, and then place it on ice for 5 minutes;
3. Add the following components in sequence:

Component	100μl
Denatured RNA	67μl
10x Capping Reaction Buffer	10μl
GTP (10 mM)	10μl
SAM (20 mM)	2.5μl
Recombinant RNase Inhibitor (40 U/μl)	2.5μl
mRNA Cap 2´-O-Methyltransferase (100 U/μl)	4μl
Vaccinia Capping Enzyme(10U/μl)	4μl

4. React at 37℃ for 30 minutes. RNA capping is completed and subsequent experiments can be conducted.

FAQ

1. What are the reasons and solutions for low cap efficiency?
   1.1. Before the capping reaction, the RNA produced by IVT should be purified to remove residual proteins, pollutants, and unbound nucleotides, and dissolved in RNase free water. RNA should not be dissolved in EDTA or other salt solutions;
   1.2. SAM will slowly degrade at room temperature and should always be placed on ice. Due to the low efficiency of N7 methylation caused by SAM degradation, it can further lead to capping failure;
   1.3. The conditions for RNA thermal denaturation can be appropriately increased by extending the heating time to 10 minutes and the cap reaction time to 60 minutes;

1.4. Some RNAs will form stable structures at the 5 'end (such as source dimers or hairpin structures), limiting the approach of capping enzymes. After analyzing the sequence, the denaturation temperature of RNA can be increased. If the 5 'end is highly structured, molecular biology techniques are needed to modify the sequence. Usually, single point mutations can be achieved in the first 5 bases of the DNA template of the transcribed RNA (non coding region)

2. How to solve the problem of white precipitate in buffer solution?

2.1. Incubate the reaction buffer at 37℃ for 5 minutes, thoroughly mix to dissolve the precipitate;

2.2. Do not store the reagent kit at -70℃.

Related products

Product Number	Product Name
GMP-M062	Vaccinia Capping Enzyme, GMP Grade
GMP-RI01	RNase Inhibitor, GMP Grade
M072	mRNA Cap 2' O Methyltransferase
DI05	DNase I solution(Bovine Pancrease)
M012	Poly(A) Polymerase
M036	Pyrophosphatase, Inorganic (yeast)
E131	T7 High Yield RNA Transcription kit
TR01	T7 RNA Polymerase
M037	Thermostable Pyrophosphatase, Inorganic