mRNA Capping Kit

Please read the manual carefully before use

Cat. No.: LC101

Storage: -20℃ for one year, avoid repeated freezing and thawing.

Description

This product contains vaccinia virus capping enzyme, mRNA cap structure 2'-O-methyltransferase and other capping reaction components, which can be used for 5' capping modification of RNA produced by T7 in vitro transcription (JT101).

In eukaryotes, after transcription to form the original mRNA, a special cap structure needs to be formed at the 5' end. This structure plays an important role in the stability, transport (exit) and translation of mRNA. The capping modification of the 5' end of RNA by the enzymatic reaction of capping enzymes is a simple and effective method: Vaccinia Capping Enzyme can attach 7-methylguanylate cap (m7Gppp, Cap0) to the 5' end of RNA to form m7Gppp5'N-mRNA (Cap0-mRNA). The mRNA Cap 2 '-O-methyltransferas uses CAP0-mRNA as substrate and SAM (S-adenosine methionine) as a methyl donor to methylate 2' -oh of the first nucleotide of cap0-mrna 5 'end adjacent to the cap structure to form Cap1-mRNA.

Cap1 structure can improve mRNA stability, which helps to enhance its expression ability in cell transfection and microinjection experiments.

This product uses vaccinia virus capping enzyme and mRNA Cap-2'-O-methyltransferase at the same time in the capping reaction, so that the capping reaction can be completed in the same reaction. The reaction product is Cap1-mRNA, and the capping efficiency can be close to 100% in the correct cap orientation .

mRNA Capping Reaction

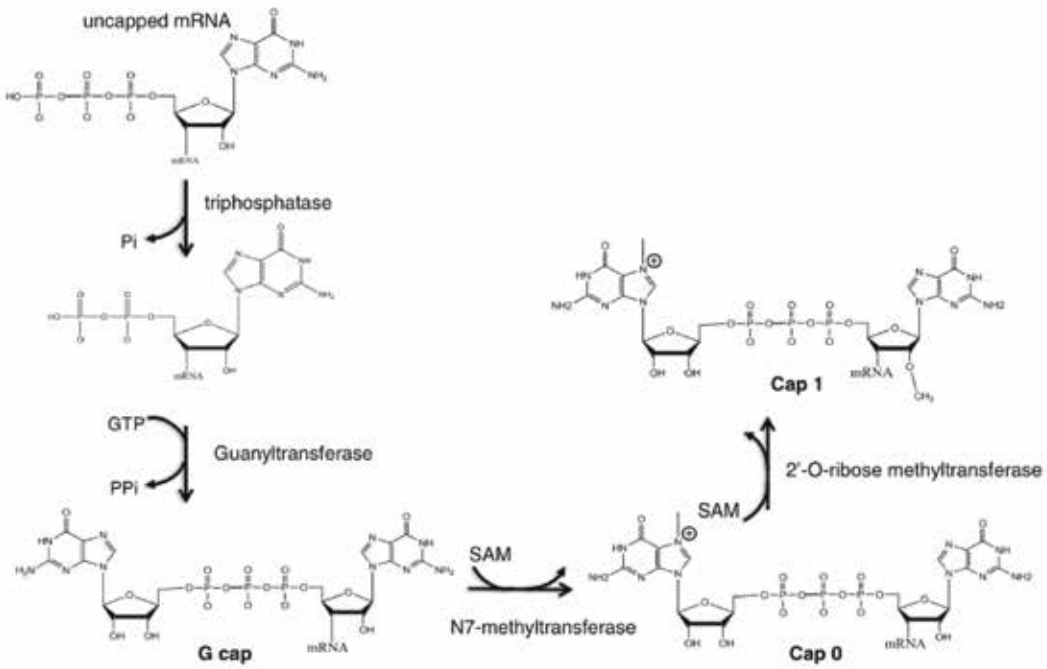

Figure 1. The mRNA capping reaction pathway involving vaccinia virus capping enzyme and mRNA Cap-2'-O-methyltransferase. Image modified from Michael Beverly, Amy Dell, et.al., Label-free Analysis of mRNA Capping Efficiency Using RNaseH Probes And LC-MS. Anal Bioanal Chem (2016).

Kit Contents (20 µl reaction)

Components	LC101-01 (25 rxns)	LC101-02 (100 rxns)
Vaccinia Capping Enzyme	20 µl	80 µl
mRNA Cap 2'-O-Methyltransferase	40 µl	160 µl
10×Capping Reaction Buffer	50 µl	200 µl
GTP (10 mM)	50 µl	200 µl
SAM (32 mM)	10 µl	40 µl
Ribonuclease Inhibitor	10 µl	40 µl
RNase-free Water	1 ml	2×1 ml

Enzyme Storage Buffer

• 20 mM Tris-HCl (pH 8.0, 25°C), 100 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.1% (v/v) Triton X-100, 50% (v/v) Glycerol.

Definition of Enzyme Activity

• Vaccinia Capping Enzyme: The amount of enzyme required to incorporate 10 pmol of GTP into an 80 nt transcript within 1 hour at 37°C was defined as 1 activity unit (Unit);

• mRNA Cap 2'-O-Methyltransferase: The amount of enzyme required to methylate 10 pmol of 80 nt capped RNA transcript in 1 hour at 37°C was defined as one unit of enzyme activity.

Procedures (this protocol is used for capping modification of ≤10 µg RNA in a 20 µl reaction , and the reaction volume can be scaled up according to experimental needs)

Preparation before the experiment: SAM reagent has poor stability at pH 7~8 and 37°C. It needs to be prepared right before use: Calculate the volume of SAM required for the reaction in advance.Dilute the 32 mM stock solution with RNase-free Water to 20 mM working solution before the reaction. The working solution should always be kept on ice to prevent SAM degradation;

(1) Take 10 µg RNA to a PCR tube and dilute it to the required volume with RNase-free Water (see the following reaction volume for details);

(2) Heat at 65°C for 10 minutes (if the structure of the 5' end of the RNA is complex, it can be extended to 60 minutes). Take it out and place it on ice for 5 minutes to complete RNA denaturation;

(3) Prepare capping reactions in PCR tubes according to the following table:

Reaction Components	Volume	Working Concentration
Denatured RNA	≤10 µg	-
10×Capping Reaction Buffer	2 µl	1×
GTP (10 mM)	2 µl	1 mM
SAM (20 mM)	0.5 µl	500 µM
Ribonuclease Inhibitor	0.4 µl	-
Vaccinia Capping Enzyme	0.8 µl	-
mRNA Cap 2'-O-Methyltransferase	1.6 µl	-
RNase-free Water	to 20 µl	-
Total Volume	20 µl	-

(4) Incubate for 1 hour at 37°C (Can be extended to 2 hours for capping RNAs <200 nt)

(5) At this point, the mRNA has been capped, and theoretically the products are all Cap1-mRNA, which can be used for downstream applications. If the capped RNA needs to be tailed, it is recommended to use TransGen's mRNA Poly (A)-Tailing Kit (Cat. No.: LA201) for 3' tailing. The capped and tailed mRNA needs to be purified before use for transfection.

Notes

• Avoid RNase contamination: Be sure to use RNase-free water, pipette tips (with filter cartridges) and centrifuge tubes. Wear lab coats and gloves during operation;

- The RNA used for the capping reaction needs to be purified and dissolved in RNase-free water, and all solutions must not contain EDTA and salt ions;
- Before the reaction, heat the RNA at 65°C to denature it to remove the secondary structure at the 5' end of the transcript. If the 5' end structure of the RNA is known to be complex, the heating time can be appropriately extended to 60 minutes to improve the capping efficiency;
- The stability of SAM reagent is poor at pH 7~8 and 37°C. It needs to be prepared right before use: Calculate the volume of SAM required for the reaction in advance.Dilute the 32 mM stock solution with RNase-free Water to 20 mM before the reaction. The working solution should always be kept on ice to prevent SAM degradation;
- The reaction volume can be scaled up or down in parallel according to the needs of the experiment. For example, a 100 µl reaction can be used to cap more than 50 µg of in vitro transcribed mRNA. The reaction volumes are as follows:

Reaction Components	Volume	Working Concentration
Denatured RNA	≤50 µg	-
10×Capping Reaction Buffer	10 µl	1×
GTP (10 mM)	10 µl	1 mM
SAM (20 mM)	2.5 µl	500 µM
Ribonuclease Inhibitor	2 µl	-
Vaccinia Capping Enzyme	4 µl	-
mRNA Cap 2'-O-Methyltransferase	8 µl	-
RNase-free Water	to 100 µl	-
Total Volume	100 µl	-

Application Example

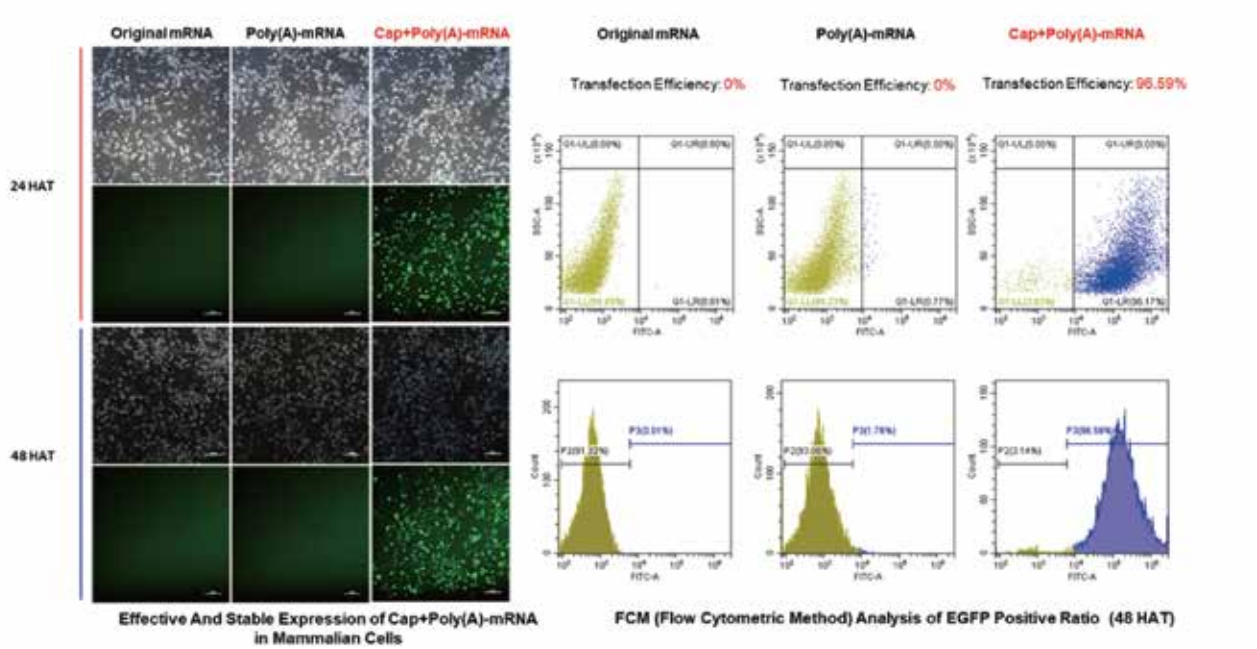

Figure 2. The capped-modified GFP-mRNA with this product can be stably and highly expressed after transfecting cells

Quality Control

Item	Standard	Method
Source	Recombinant *E. coli*	
Appearance	Transparent and clear	Visual inspection
Activity	Indicated value	Capping efficiency assay
Purity	≥95%	SDS-PAGE or SEC-HPLC
Endonuclease residues	Not detected	When 10 U vaccinia virus capping enzyme/50 U 2'-O-methyltransferase and 1 μg λDNA are incubated at 37°C for 16 hours, the DNA electrophoresis bands do not change
Exonuclease residues	Not detected	When 10 U vaccinia virus capping enzyme/50 U 2'-O-methyltransferase and 1 μg λ-Hind III digest DNA are incubated at 37°C for 16 hours, the DNA electrophoresis bands do not change
Nickase activity	Not detected	When 10 U vaccinia virus capping enzyme/50 U 2'-O-methyltransferase and 1 μg pBR322 plasmid are incubated at 37°C for 16 hours, the DNA electrophoresis bands do not change
RNase residue	Not detected	When 10 U vaccinia virus capase/50 U 2'-O-methyltransferase are incubated with 1.6 μg MS2 RNA at 37°C for 4 hours, the RNA electrophoresis band does not change
Bacterial endotoxin content	≤10 EU/mg	Chinese Pharmacopoeia 2020 Edition Part 4 Gel Limit Experiment (General Chapter 1143)
E. coli DNA residues	≤1 copy/U	Quantitative Real-time PCR
Mycoplasma detection	Negative	Mycoplasma Detection Kit

For research use only, not for clinical diagnosis.

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