

mRNex® Vaccinia Capping System

Cat. no. LD301007

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Product Information

Product	mRNex® Vaccinia Capping System
Catalog No.	LD301007
Storage conditions	Stored at -25~-15°C, valid for 1 year.
Size	50T/100T/500 T

2. Product Description

In eukaryotic organisms, after transcription, mRNA undergoes post-transcriptional modifications that result in the formation of a special structure at the 5' end, known as the cap structure. This cap structure plays an important role in the stability, transport, and translation of mRNA. The vaccinia virus capping enzyme is an effective enzyme that catalyzes the formation of the cap structure. It consists of two subunits, D1 and D12, and exhibits RNA triphosphatase (TPase) activity, guanylyltransferase (GTase) activity, and guanine N7-methyltransferase (N7MTase) activity. The mRNA capping process catalyzed by the vaccinia virus capping enzyme is illustrated in Figure 1, where a 7-methylguanosine cap structure (m7Gppp) is added to the 5' end of mRNA, forming Cap 0 (Figure 2).

The ribose 2'-hydroxyl group at the +1 position of the mRNA can also be methylated, resulting in Cap1, a structure found in human mRNA. Cap1 is not only involved in translation initiation but also serves as a marker for self RNA in the innate immune system against foreign RNA. A 2'-O-methyltransferase adds a methyl group to the 2'-OH position of the nucleotide at the mRNA +1 position, ultimately forming the most mature Cap1 structure (Figure 3).

This product is mainly used for capping RNA in vitro after transcription to form mRNA with a Cap1 structure.

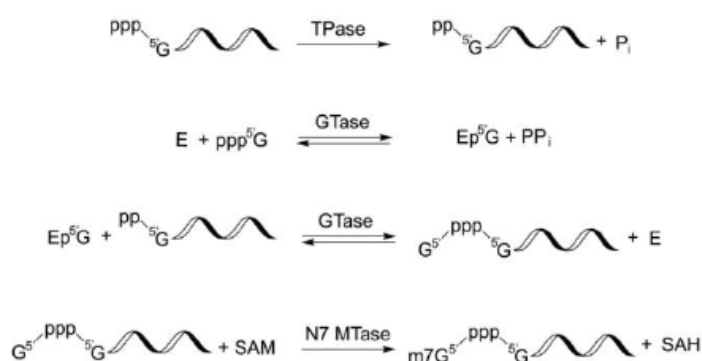


Figure 1. The formation process of Cap 0

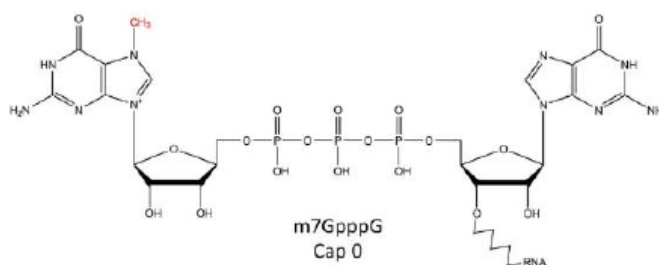


Figure 2. mRNA with Cap 0.

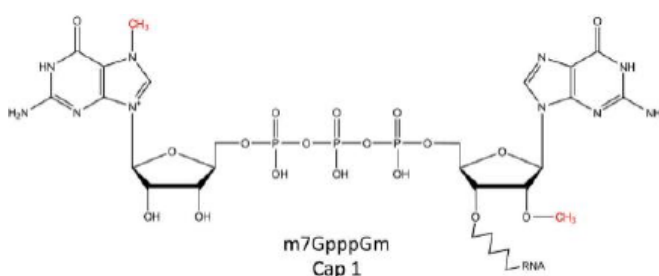


Figure 3. mRNA with Cap 1.

3. Product Component

Cat No.	Component	Size		
		50T	100T	500T
LD301007-A	10×Capping Buffer	100µL	200µL	1000µL
LD301007-B	Vaccinia Capping Enzyme (10U/µl)	50µL	100µL	500µL
LD301007-C	2-O-methyltransferase (50U/µl)	50µL	100µL	500µL
LD301007-D	RNase inhibitor (40U/µl)	25µL	50µL	250µL
LD301007-E	SAM (32mM)	12.5µL	25µL	125µL
LD301007-F	GTP (10mM)	50µL	100µL	500µL
LD301007-G	DEPC-H2O	2500µL	5000µL	25mL

Additional materials prepared by users: transcribed RNA, RNA purification reagents.

Note: RNA can be synthesized using LD201001 (T7 High Yield RNA Synthesis Kit).

4. Instructions for Use

4.1. Enzyme-mediated capping reaction (using a 20µL reaction system as an example):

4.1.1. Take 10 µg of RNA and dilute it to 14µL with DEPC-H₂O.

4.1.2. Heat the RNA at 65°C for 5 minutes, then immediately place it on ice for 5 minutes.

4.1.3. Sequentially add the following components:

Component	Volume(μL)	Final concentration
Denatured RNA	10μg	0.5μg/μl
10×Capping Buffer	2	1×
GTP (10mM)	1	0.5mM
SAM (32mM)	0.5	0.8mM
RNase inhibitor (40U/μl)	0.5	1U/μl
Vaccinia Capping Enzyme (10U/μl)	1	0.5U/μl
2'-O-methyltransferase (50U/μl)	1	2.5U/μl

5. Notes

- 5.1. The capping efficiency is influenced by the RNA 5' end sequence and structure. Therefore, it is recommended to open the secondary structure of the RNA by heat denaturation before the capping reaction to make the RNA more accessible for capping. The general heat denaturation conditions are 65°C for 5 minutes, followed by immediate placement on ice, but the denaturation time can be appropriately extended depending on the complexity of the 5' end sequence and structure, as well as the capping reaction volume.
- 5.2. The SAM concentration in this kit is 32mM. If the sample volume is too small, the 32mM stock solution can be diluted with DEPC-H₂O to make a working solution with a concentration of 8mM or lower, depending on the required amount. Since SAM is unstable, the dilution process should be performed on ice, and the amount of dilution should be equal to the amount used. It is not recommended to dilute the stock solution to a low-concentration working solution all at once.
- 5.3. If only RNA with a Cap0 structure is needed, 2'-O-methyltransferase can be omitted from the reaction system.
- 5.4. The capping reaction can generally be completed within 30 minutes. The duration can be adjusted based on the RNA 5' end sequence, length, and reaction volume.
- 5.5. This product is for research use only.
- 5.6. For your safety and health, please wear a lab coat and disposable gloves while handling the reagents.