Vaccinia Virus Capping Enzyme

Cat.No.:HYJ231

Product Description

Vaccinia virus capping enzyme, derived from a recombinant Escherichia coli strain carrying the vaccinia virus MR, consists of two subunits, D1R and D12L, and possesses three enzymatic activities: RNA triphosphatase, guanylyltransferase, and guanine methyltransferase. Vaccinia virus capping enzyme is an efficient enzyme that catalyzes the formation of a cap structure, specifically adding a 7-methylguanylate cap structure (m7Gppp, Cap 0) to the 5' end of RNA. The cap structure (Cap 0) plays a crucial role in the stability, transport, and translation of eukaryotic mRNA. Enzymatic RNA capping is an effective and simple method that can significantly improve the stability and translational capacity of RNA for in vitro transcription, transfection, and microinjection.

Components

Components	2KU	10KU	100KU	1000KU
Vaccinia Virus Capping Enzyme (10 U/μL)	0.2 mL	1 mL	10 mL	100 mL
10× Capping Buffer	0.4 mL	2×1mL	2×10 mL	2×100mL

Storage

Store at -25 to -15°C. Valid for 2 years (avoid repeated freezing and thawing).

Storage buffer

20 mM Tris-HCl (pH 8.0), 100 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 0.1% Triton X-100, 50% glycerol.

Activity Definition

One unit (U) of activity is defined as the amount of enzyme required to completely incorporate 10 pmol of GTP into an 80-nt transcript within 1 h at 37°C.

Quality Control

1. **Exonuclease activity:** When 10 U of this product was reacted with 1 μg of λ-Hind III digested DNA at 37°C for 16 hours, the electrophoretic band of the DNA remained unchanged.
2. **Endonuclease activity:** When 10 U of this product was reacted with 1 μg of λ DNA at 37°C for 16 hours, the electrophoretic band of the DNA remained unchanged.

3. **Nickase activity:** When 10 U of this product was reacted with 1 μg of pBR322 at 37°C for 16 hours, the electrophoretic band of the DNA remained unchanged.
4. **RNase activity:** When 10 U of this product was reacted with 1.6 μg of MS2 RNA at 37°C for 4 hours, the electrophoretic band of the RNA remained unchanged.
5. **Residual E. coli DNA:** Residual nucleic acid in 10 U of this product was detected by TaqMan qPCR specific for E. coli 16s rDNA, and the E. coli genome residue was ≤1. Copy;
6. **Endotoxin:** LAL-Test, Chinese Pharmacopoeia 2020 Edition Part IV Gel Limit Test General Rules (1143) , bacterial endotoxin content ≤ 10 EU/mg.

Reaction system and conditions

1. Capping Reaction (20 μL Reaction System)

This protocol is suitable for capping 10 μg of RNA (≥ 100 nt) and can be scaled up as needed:

1) Transfer 10 μg of RNA to a 1.5 mL microcentrifuge tube and dilute to 15 μL with nuclease-free water;
2) Heat at 65°C for 5 minutes, then remove from the tube and place on ice for 5 minutes to complete denaturation;
3) Prepare the capping system according to the table below.

Components	Volume
Denatured RNA(≤10 μg, length ≥100 nt)	15 uL
10× Capping Buffer*	2 uL
GTP (10 mM)	1 uL
SAM (2 mM)	1 uL
Vaccinia Virus Capping Enzyme (10 U/μL)	1 uL

*10× Capping Buffer: 0.5 M Tris-HCl, 50 mM KCl, 10 mM MgCl2, 10 mM DTT (25°C, pH 8.0)

4) Incubate at 37°C for 30 min. RNA capping is complete and subsequent experiments can be performed.

2. 5'-End Labeling Reaction (20 μL Reaction System)

This protocol is suitable for labeling RNA with a triphosphate at the 5' end and can be scaled up as needed. The labeling efficiency is affected by the molar ratio of RNA to GTP in the reaction system and the GTP content in the RNA sample:

1) Transfer an appropriate amount of RNA to a 1.5 mL centrifuge tube and dilute to 14 μL with nuclease-free water;

2) Heat at 65°C for 5 minutes, then remove from the tube and place on ice for 5 minutes to complete denaturation;
3) Prepare the 5'-end labeling reaction system according to the following table:

Components	Volume
Denatured RNA	14 uL
10× Capping Buffer	2 uL
GTP mix*	2 uL
SAM (2 mM)	1 uL
Vaccinia Virus Capping Enzyme (10 U/μL)	1 uL

* GTP mix contains GTP and a small amount of labeling agent. Refer to Note 3 for the GTP concentration.

4) Incubate at 37°C for 30 minutes. RNA 5' end labeling is complete and subsequent experiments can be performed.

Applications

1. Capping of mRNA before in vivo or in vitro translation;
2. 5' end labeling of mRNA.

Notes

1. Heat-treat the RNA before the reaction to remove secondary structures at the 5' end of the transcript. If the 5' end of the RNA is known to have a complex structure, extend the reaction time to 60 minutes to improve capping efficiency.
2. The RNA used for the capping reaction should be purified and dissolved in nuclease-free water before use. The solution should be free of EDTA and salts.
3. In the 5' end-labeling reaction, the GTP stock solution should be diluted to 1-3 times the molar concentration of the mRNA in the reaction.
4. The reaction volume can be scaled up or down based on actual needs.