Product Manual

Product Name	Vaccinia Capping Enzyme（GMP-grade）
Source	Recombinant expression in Escherichia coli
Catalog Number	CSB-DEM082
Physical Form	Liquid
Enzyme activity	10 U/μL
Storage Conditions	-20±5°C
Storage Buffer	20mM Tris-HCl pH 8.0, 100 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 0.1% Triton X-100, 50% glycerol
Activity definition	The amount of enzyme required to incorporate 10 pmol GTP (α-32P) into an 80 nucleotide (80nt) transcript in one hour at 37°C.
Shelf Life	12 months

Product Description

In eukaryotes, mRNA undergoes post-transcriptional modifications to form a special structure at the 5' end, known as the cap structure. This structure plays a crucial role in mRNA stability, transport, and translation. Vaccinia capping enzyme, derived from cowpox virus, is an effective enzyme that catalyzes the formation of the cap structure. It consists of two subunits, D1 and D12, and possesses RNA triphosphatase activity, guanylyltransferase activity, and guanine-N7 methyltransferase activity. It can connect a 7-methylguanosine cap structure (m7Gppp) to the 5' end of RNA (m7Gppp5'N). Vaccinia capping enzyme, in the presence of suitable concentrations of capping buffer, guanosine triphosphate (GTP), S-adenosylmethionine (SAM), and other conditions, can cap RNA within one hour while ensuring the correct orientation.

This product is provided in sterile liquid form and can be used for capping reactions of mRNA before in vitro/in vivo translation or 5' end labeling reactions of mRNA.

Product Components

Label	Components	Specifications		
1	Vaccinia Capping Enzyme（GMP-grade）	5KU	10KU	50KU
2	10×Capping Reaction Buffer	0.2mL	0.4mL	2mL

Operating instructions

This step is suitable for capping reactions of 10 μg RNA (≥ 100 nt) with a reaction system of 20 μL. It can be scaled up according to experimental needs.

1. Transfer 10 μg RNA to a 1.5 mL centrifuge tube and dilute with nucleic acid-free water to 9.5 μL.

2. Heat at 65°C for 5 minutes.

3. Place the centrifuge tube on ice for 5 minutes.

4. Add the following components sequentially:

Components	Volume
Denatured RNA	9.5 μL
10×Capping Buffer	2.0 μL
Murine RNase inhibitor(40 U/μL)	0.5 μL
GTP (10 mM)	1.0 μL
SAM (10 mM, fresh)	1.0 μL
Vaccinia Capping Enzyme (10 U/μL)	5.0 μL
Cap 2′-O-Methyltransferase (50 U/μL)	1.0 μL

5. Incubate at 37°C for 2 hours.

6. RNA capping is complete and can be used for subsequent experiments.

Notes

1. The extracted RNA should be purified and reconstituted using nucleic acid-free water.

2. The RNA solution needs to be heated before adding the enzyme to remove secondary structures at the 5' end.

3. For RNA with known 5' end structures, the reaction time can be extended to 4 hours to improve capping efficiency.

4. In the 5' end labeling reaction system, the GTP stock solution should be diluted to 1-3 times the molar concentration of mRNA in the reaction system.