Cap1 capping system

Cat.No.:HYJ246

Product Description

The enzymatic capping kit contains vaccinia capping enzyme, mRNA Cap 2′-O-methyltransferase, and other capping components. It can add cap0 or cap1 structures to the 5' end of uncapped mRNA. This structure is identical to the natural cap structure in eukaryotic organisms and can significantly reduce the immunogenicity of exogenous mRNA, enhance its stability, and improve translation efficiency. The vaccinia virus capping enzyme in this kit is derived from recombinant Escherichia coli carrying the vaccinia virus MR. This enzyme consists of two subunits, D1R and D12L, and possesses three enzymatic activities: RNA triphosphatase, guanylyltransferase, and guanine methyltransferase. The D1 subunit performs the functions of RNA triphosphatase and guanylyltransferase, while the D12 subunit functions as a guanine methyltransferase, specifically adding a 7-methylguanylate cap structure (Cap 0) to the 5' end of RNA.

The mRNA Cap 2′-O-methyltransferase is derived from a recombinant Escherichia coli strain carrying the vaccinia virus mRNA Cap 2′-O-methyltransferase gene. This enzyme uses S-adenosylmethionine (SAM) as a methyl donor to methylate capped RNA (Cap 0), forming the Cap 1 structure. This adds a methyl group to the 2'-O position of the first nucleotide immediately preceding the cap structure at the 5' end of the RNA.

The Cap 1 capping system catalyzes four enzymatic reactions: ①RNA triphosphatase cleaves RNA 5'-triphosphate into diphosphate. ② RNA guanylyltransferase attaches GTP to the 5'-diphosphate of RNA N1. ③ Guanine 7-methyltransferase, using S-adenosylmethionine as a cofactor, catalyzes the methylation of the 7-nitrogen of guanine. ④ mRNA Cap 2'-O-methyltransferase adds a methyl group to the 2'-O position of the first nucleotide immediately preceding the cap structure at the 5' end of Cap 0-RNA.

This kit can cap 50 μg of RNA in a single reaction, allowing for scale-up to produce milligram quantities of RNA. Capping enhances mRNA translation efficiency, significantly improving mRNA expression in transfection and microinjection experiments.

Components

Components	Concentration	50T
Vaccinia Capping Enzyme	10 U/μL	250 μL
mRNA Cap 2′ -O-Methyltransferase	50 U/μL	250 μL
SAM	32 mM	50 μL
GTP	100 mM	50 μL
Recombinant RNase Inhibitor	40 U/μL	150 μL
10×Capping Buffer	/	500 μL

Storage

Store at -25 to -15°C. Valid for 1 years.

Storage buffer

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

Activity Definition

Vaccinia Capping Enzyme: 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 hour at 37°C.

mRNA Cap 2′-O-Methyltransferase: One unit (U) of activity is defined as the amount of enzyme required to methylate 10 pmol of an 80-nt capped RNA transcript within 1 hour at 37°C.

Instructions

This system is suitable for capping reactions of 50 μg RNA and can be scaled up according to experimental needs.

1. Calculate the volume of SAM required for the reaction before the experiment. Dilute 32 mM SAM with RNase-Free Water to a working concentration of 4 mM SAM.
2. Transfer 50 μg of RNA to a centrifuge tube and dilute to 71.5 μL with nuclease-free water.
3. Heat at 65°C for 5 minutes, then place on ice for 5 minutes to complete denaturation.
4. Prepare the capping system according to the following table:

Components	Volume
Denatured capped RNA	71.5 μL (50μg)
10×Capping Buffer	10 μL

GTP（100 mM）	1 μL
SAM（4 mM）	5 μL
Recombinant RNase Inhibitor	2.5 μL
Vaccinia Capping Enzyme	5 μL
mRNA Cap 2′-O-Methyltransferase	5 μL
Total volume	Up to 20 μL

5. Incubate at 37°C for 1 h (for target RNA fragments less than 200 nt in length, the incubation time can be increased to 2 h).

Notes

1. To avoid RNase contamination, use RNase-free water, pipette tips, and centrifuge tubes.
2. Before starting the reaction, RNA must be purified and dissolved in nuclease-free water. All solutions must be free of EDTA and salt ions.
3. Before the reaction, ensure that the 10× Capping Buffer is clear. If a white precipitate forms, incubate the reaction buffer at 37°C for 5 minutes and then mix thoroughly to dissolve the precipitate before use.
4. Before the reaction, it is recommended to heat the sample RNA at 65°C for 5 minutes to remove secondary structures at the 5' end of the transcript. If the 5' end of the transcript is complex, the heating time can be extended to 10 minutes.
5. 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.
6. SAM reagent has poor stability at pH 7–8 and 37°C and should be prepared fresh. Furthermore, to prevent SAM degradation, the working solution should be kept on ice. SAM degradation can lead to low N7 methylation efficiency, which in turn reduces the capping rate.
7. The reaction volume can be scaled up or down based on actual needs.
8. For your safety and well-being, please wear a lab coat and disposable gloves during operation.
9. This reagent is for research and production use only and is not intended for direct human use.