mRNA Cap2'-O-Methyltransferase

Cat.No.:HYJ232

Product Description

mRNA Cap 2′-O-Methyltransferase (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 adds a methyl group to the 2′-O position of the first nucleotide immediately preceding the cap structure at the 5′ end of RNA. It can also methylate capped RNA (Cap 0) using S-adenosylmethionine (SAM) as a methyl donor, thereby forming a Cap 1 structure. The Cap 1 structure enhances mRNA translation efficiency and can therefore help improve mRNA expression in transfection and microinjection experiments. This enzyme requires RNA with a 7-methylguanosine cap structure (m7GpppN) as a substrate and cannot be used with RNA containing pN, ppN, pppN, or GpppN at the 5' end. Capped RNA can be prepared by in vitro transcription using a capping analog or enzymatically capped using Vaccinia Capping Enzyme.

Components

Components	2.5KU	10KU	50KU	500KU	5000KU
mRNA Cap2'-O-Methyltransferase (50 U/μL)	0.05 mL	0.2 mL	1 mL	10 mL	100 mL
10× Capping Buffer	0.1 mL	0.4 mL	2×1 mL	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 8.0，25°C), 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 methylate 10 pmol of 80-nt capped RNA transcript in 1 hour at 37°C.

Quality Control

1. **Exonuclease activity:** When 50 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 50 U of this product was reacted with 1 μg of λDNA at 37°C for 16 hours, the electrophoretic band of the DNA remained unchanged.
3. **Nickase activity:** When 50 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 50 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 50 U of this product was detected by E. coli 16s rDNA-specific TaqMan qPCR, and the residual E. coli genome 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. Dilute the appropriate amount of capped RNA to 16 μL with RNase-free water.
2. Heat the diluted RNA at 65°C for 5 minutes, then place on ice for 5 minutes.
3. Prepare the reaction mixture according to the following table (applicable to methylation reactions of up to 10 μg of capped RNA):

Components	Volume
Denatured capped RNA	16 uL
10× Capping Buffer*	2 uL
SAM (4 mM)	1 uL
mRNA Cap2'-O-Methyltransferase (50 U/μL)	1 uL
ddH_2O	Up to 20 uL

*10× Capping Buffer: 500 mM Tris-HCl (pH 8.0, 25°C), 50 mM KCl, 10 mM $MgCl_2$, 10 mM DTT.

4. Incubate at 37°C for 1 hour (for RNA targets less than 200 nt in length, increase the incubation time to 2 hours).

Applications

Improve mRNA expression in microinjection and transfection experiments.

Notes

1. Before starting the reaction, RNA must be purified and dissolved in nuclease-free water. All solutions must be free of EDTA and salt ions.

2. 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.
3. SAM reagent is less stable at pH 7–8 and 37°C and should be prepared immediately before use. You can pre-calculate the required amount of SAM and dilute the 32 mM SAM stock solution to a 4 mM working solution before the reaction. To prevent SAM degradation, the working solution should be kept on ice.
4. For your safety and well-being, please wear a lab coat and disposable gloves during this procedure.