mRNA Cap 2'-O-Methyltransferase (50 U/μl)

DD4110PCEN

Version 22.1

Product Description

The product mRNA Cap 2'-O-Methyltransferase (2'-oxy-methyltransferase) is a recombinant methyltransferase encoded by vaccinia virus DNA and expressed in *E. coli*. The enzyme employs S-adenosylmethionine (SAM) as the methyl donor and adds a methyl group at the 2'-O site of the first nucleotide near the cap structure (cap 0) at the RNA 5' end to form mRNA with a cap 1 structure, which can enhance the translation efficiency of mRNA and reduce the immunogenicity of the mRNA construct, thus improving the expression of the encoded proteins following mRNA transfection. 2'-O-methyltransferase specifically recognizes the 7-methylguanosine cap structure (m7Gppp, cap 0) and does not act on RNAs with pN, ppN, pppN, or GpppN at the 5' end. RNA with the cap 0 structure can be prepared using the Vaccinia Capping Enzyme (Vazyme #DD4109PCEN).

Components

Component	DD4110PCEN-01 (50 KU)	DD4110PCEN-02 (250 KU)	DD4110PCEN-03 (1 MU)
mRNA Cap 2'-O-Methyltransferase (50 U/μl)	1 ml	5 ml	20 ml

Storage

Store at -30 ~ -15℃ and transport at ≤ 0℃.

Product Information

Product Name	mRNA Cap 2'-O-Methyltransferase
Source	Recombinant *E. coli*
Activity	50 U/μl
Unit Definition	One unit is defined as the amount of enzyme required to methylate 10 pmol of 80-nt oligonucleotide transcripts with the m7Gppp cap structure within 1 hour at 37℃, pH 8.0.
Capping Buffer (1×)	50 mM Tris-HCl (25℃, pH 8.0), 5 mM KCl, 1 mM $MgCl_2$, 1 mM DTT
Storage Buffer	50 mM Tris-HCl (25℃, pH 8.0), 100 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.1% Triton X-100, 50% glycerol
Storage Conditions	-30 ~ -15℃

Reaction conditions: reaction at 37℃ for 1 h

Application

To improve the efficiency of mRNA translation and expression following transfection and reduce the in vivo immunogenicity of mRNA

Recommended Reaction Systems

For Multi-step Capping

Component	Volume	Final Concentration
10 × Capping Buffer	2 μl	1 ×
mRNA Cap 2'-O-Methyltransferase (50 U/μl)	1 μl	2.5 U/μl
SAM (4 mM)	1 μl	0.2 mM
Denatured Cap0 RNA	10 μg	500 ng/μl
RNase-free ddH_2O	Up to 20 μl	-

For One-Step Capping

Component	Volume	Final Concentration
10 × Capping Buffer	2 μl	1 ×
Vaccinia Capping Enzyme (10 U/μl)	1 μl	0.5 U/μl
mRNA Cap 2'-O-Methyltransferase (50 U/μl)	1 μl	2.5 U/μl
GTP (10 mM)	1 μl	0.5 mM
SAM (4 mM)	1 μl	0.2 mM
Denatured RNA	10 μg	500 ng/μl
RNase-free ddH_2O	Up to 20 μl	-

Notes

1. For research use only. Not for use in diagnostic procedures.
2. The efficiency of the capping reaction is affected by the 5' end structure of RNA, so it is recommended to break up the higher-order 5' end structures of RNA by thermal denaturation (heating at 65℃ for 5 min and keeping on ice for 5 min). The denaturation conditions can be adjusted according to the complexity of the 5' end structure of RNA, and this step can be omitted if there is no higher-order structure at the 5' end.
3. The capping reaction can generally be completed within 60 min. In case of complex 5' end structure or short RNA length (≤ 200 nt), the reaction time can be extended to 120 min.
4. SAM is unstable at 37℃, pH 7 - 8 and should be freshly prepared prior to reaction. To avoid SAM degradation, the working solution should be kept on ice.
5. Murine RNase Inhibitor Neo (Vazyme #DD4117PAEN)can be added to the reaction system to prevent RNase contamination. A concentration of 1 - 2 U/μl is recommended.
6. Cap 1 RNA obtained with this product can be added with the Poly(A) sequence at the 3' end with Poly(A) polymerase to form complete mRN A for subsequent transfection or in vitro translation experiments.