mRNA Cap 2´-O-Methylt ransferase GMP-grade

Product Description

2'-O-methyltransferase (2OM) is a methyltransferase encoded by the vaccinia virus DNA and expressed in recombinant *E. coli*. It is a methyltransferase that further modifies the Cap0 cap structure into the Cap1 cap structure. Using S-adenosyl methionine (SAM) as the methyl donor, this enzyme adds a methyl group to the 2'-O position of the first nucleotide immediately adjacent to the Cap0 cap structure at the 5' end of the RNA, thereby generating an mRNA bearing the Cap1 structure. This modification can further reduce the intrinsic immunogenicity of the mRNA and enhance the expression level of the encoded protein post-transfection.

Components

Components	GMP-BP-E06-5K	GMP-BP-E06-50K	GMP-BP-E06-500K
	5 KU	50 KU	500 KU
mRNA Cap 2´-O-Methyltransferase GMP-grade (50 U/μL)	0.1 mL	1 mL	10 mL
10× Capping Buffer	0.2 mL	2 mL	20 mL
SAM (32 mM)	20 μL	0.2 mL	2 mL

Storage

Store at -20±5℃.

Product Information

Product Name	mRNA Cap 2´-O-Methyltransferase GMP-grade
Source	Recombinant *E.coli*
Activity	50 U/μL
Unit Definition	One unit of enzyme activity is defined as the amount of enzyme required to incorporate 10 pmol of methyl groups into Cap0 RNA in 1 hour at 37℃.
Capping Buffer	500 mM Tris-HCl (pH 8.0), 50 mM KCl, 10 mM $MgCl_2$, 10 mM DTT
Storage Buffer	20 mM Tris-HCl (pH 8.0), 0.1 mM EDTA, 1 mM DTT, 100 mM NaCl,0.1% (v/v) Triton X-100, 50% (v/v) glycerol

Quality control

1. Solution appearance: clear and transparent, free of visible particulate matter.
2. Activity>50 U/μL.
3. Protein purity≥95%.
4. Free of exogenous DNase, RNase, exonuclease, and endonuclease activity.
5. Residual host-cell DNA: ≤100 pg/mg.
6. Residual host-cell protein: <50 ppm.
7. Heavy metals<10 ppm.
8. HBV, HCV, HIV, and mycoplasma: not detected.
9. Bacterial endotoxin: <5 EU/mL.
10. pH 7.5-8.5.

Stepwise Capping System

Components	Volume
10× Capping Buffer	2 μL
SAM (4 mM)	1 μL
mRNA Cap 2´-O-Methyltransferase GMP-grade (50 U/μL)	1 μL
RNase Inhibitor GMP-grade (40 U/μL)	0.5 μL
Denatured Cap0 RNA	50 pmol
RNase-free ddH_2O	To 20 μL

One-step Capping system

Components	Volume

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10× Capping Buffer	2 μL
GTP Solution (10 mM)	1 μL
SAM (4 mM)	1 μL
Vaccinia Capping Enzyme GMP-grade (10 U/μL)	1 μL
mRNA Cap 2´-O-Methyltransferase GMP-grade (50 U/μL)	1 μL
RNase Inhibitor GMP-grade (40 U/μL)	0.5 μL
Denatured RNA	50 pmol
RNase-free ddH_2O	To 20 μL

*Incubate at 37℃ for 1 hour.

Notes

1. Capping efficiency is influenced by the secondary structure at the RNA 5' end. It is recommended to denature the RNA prior to the reaction by heating at 65℃ for 5 min followed by immediate cooling on ice for 5 min.
2. The capping reaction is generally complete within 1 h. For RNA with complex 5'-end secondary structure or short RNA (≤200 nt), the incubation time may be extended to 2 hours.
3. SAM is a high-energy methyl donor with limited stability. Thaw SAM on ice and add it to the reaction mixture while keeping on ice throughout.
4.Please mix thoroughly before use. Avoid repeated freezing and thawing.