KACTUS

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mRNA Cap 2'-O-Methyltransferase, GMP-Grade

Catalog #GMP- MEH-VE101

Storage Condition -20°C ± 5°C for 24 months. Avoid repeated freeze/thaw cycles.
Form Liquid
Source An *E. coli* strain that carries the gene for Vaccinia mRNA Cap 2´-O-Methyltransferase
Storage Buffer 20mM Tris-HCl, 100mM NaCl, 50%Glycerol, 0.1mM EDTA, 1mM DTT, 0.1%Triton X-100, pH8.0(±0.05)
Concentration 50U/µL
Unit Definition One unit is defined as the amount of enzyme required to methylate 10 pmoles of 80 nt long capped RNA transcript in 1 hour at 37°C.

Product Contents

- mRNA Cap 2'-O-Methyltransferase (50U/µL)
- 10X Capping Buffer (0.5M Tris-HCl, 50mM KCl, 10mM $MgCl_2$, 10mM DTT, pH 8.0

Product Description

mRNA Cap 2'-O-Methyltransferase adds a methyl group at the 2'-O position of the first nucleotide adjacent to the cap0 structure at the 5' end of RNA to obtain a cap1 structure. The enzyme utilizes S-adenosylmethionine (SAM) as a methyl donor to methylate capped RNA (cap-0). mRNA Cap 2'-O-Methyltransferase requires RNA with an m7GpppN cap as substrate. Vaccinia Capping Enzyme, GMP grade (GMP-VCS-VE101) can be used together with mRNA Cap 2'-O-Methyltransferase to generate cap-1 mRNA in a single reaction consisting of m^7Gppp-Capping and 2'-O-Methylation. Cap-1 RNA structure may help with RNA evasion of innate immune response in some cell types and promote translation *in vivo*.

Applications

2´-O-methylation of capped *in vitro* transcripts

Quality Control Statement

This product has been filed with the FDA Drug Master Files and is assigned DMF #038029. KACTUS manufactures this product according to GMP guidelines and performs stringent quality control testing before release. The production is antibiotic- and animal-free.

Quality Control Release Criteria

Assay	Criteria
Activity (In-house method)	≥ 67.7kU/mL
Purity (SEC-HPLC)	≥ 95%
Residual Nickel Salt	≤ 10 ppm
Endotoxin	≤ 5EU/mL
Residual DNase	Negative
Residual RNase	Negative
Residual Protease	Negative
Residual Host Cell Protein	≤ 20ng/mg
Residual Host Cell DNA	≤ 100pg/mg
Residual Heavy Metal	≤ 10 ppm
Bioburden	≤ 1CFU/10mL

Protocol for 2'-O-Methylation of m7Gppp capped RNA

1. Dilute capped RNA (up to 10µg) with RNase-free water to a final volume of 16µL in a 1.5mL tube.
2. Incubate at 65°C for 5 min.
3. Place the tube on ice for 5 min.
4. Prepare the following reaction mixture in the specified order in the table below.

Reagent	Quantity
Denatured capped RNA	16µL
10X Capping Buffer	2µL
SAM (4mM)	1µL
mRNA Cap 2'-O-Methyltransferase (50U/µL)	1µL

5. Incubate at 37°C for 1 hr. For RNA less than 200 nt long, increase incubation time to 2 hrs.
6. RNA is now in cap-1 structure and ready for use for downstream applications. Purify RNA if needed.

Notes

- The reaction can be scaled up as needed.
- RNA used for capping reactions should be purified prior to use and resuspended in nuclease-free water. EDTA should not be present, and the solution should be free of salts.
- The addition of 0.5 µL Murine RNase Inhibitor can help enhance the stability of RNA.
- Heating before capping reaction helps remove secondary structure on 5' end of RNA. Heating time can be extended to 10min with known highly structured 5'ends.
- SAM is unstable at pH 7–8, 37°C and should be mixed fresh prior to starting the reaction.

One-step m^7Gppp-Capping and 2'-O-Methylation

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1. Dilute uncapped RNA (up to 10µg) with RNase-free water to a final volume of 14µL in a 1.5mL tube.
2. Incubate at 65°C for 5 minutes.
3. Place the tube on ice for 5 minutes.
4. Prepare the following reaction mixture in the specified order in the table below.

Reagent	Quantity
Denatured uncapped RNA	14µL
10X Capping Buffer	2µL
GTP (10mM)	1µL
SAM (4mM)	1µL
Vaccinia Capping Enzyme (10U/µL)	1µL
mRNA Cap 2'-O-Methyltransferase (50U/µL)	1µL

5. Incubate at 37°C for 1 hour. For RNA less than 200 nt long, increase incubation time to 2 hrs.
6. RNA is now in cap-1 structure and ready for use for downstream applications. Purify RNA if needed.

Notes

- The reaction can be scaled up as needed.
- RNA used for capping reactions should be purified prior to use and resuspended in nuclease-free water. EDTA should not be present, and the solution should be free of salts.
- The addition of 0.5 µL Murine RNase Inhibitor can help enhance the stability of RNA.
- Heating before capping reaction helps remove secondary structure on 5' end of RNA. Heating time can be extended to 10min with known highly structured 5'ends.
- SAM is unstable at pH 7–8, 37°C and should be mixed fresh prior to starting the reaction.