KACTUS

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Vaccinia Capping Enzyme, GMP-Grade

Catalog #GMP- VCS-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 capping enzyme
Storage Buffer 20mM Tris-HCl, 100mM NaCl, 50%Glycerol, 0.1mM EDTA, 1mM DTT, 0.1%Triton X-100, pH8.0
Concentration 10U/µL
Unit Definition One unit is defined as the amount of enzyme required to incorporate 10pmol of (α^{32}P) GTP into an 80 nt transcript in 1 hour at 37°C.

Product Contents

- Vaccinia Capping Enzyme (10U/µL)
- 10X Capping Buffer (0.5M Tris-HCl, 50mM KCl, 10mM MgCl2, 10mM DTT, pH 8.0)

Product Description

Vaccinia Capping Enzyme is composed of two subunits (D1 and D12) that executes all three steps in m^{7}GpppRNA synthesis. The D1 subunit has domains for RNA triphosphate (TPase), guanylyltransferase (GTase), and guanine-N7-methyltransferase (MTase), which catalyzes the addition of a complete Cap-0 structure, m7Gppp5'N to 5' triphosphate RNA. Capped mRNA help improve mRNA stability for *in vitro* translation, transfection, and microinjection. The use of Vaccinia Capping Enzyme in the same reaction with mRNA Cap 2'-O-Methyltransferase (#GMP-MEH-VE101) generates Cap-1 mRNA.

Applications

- Labeling 5' end of mRNA
- Enzymatic capping of *in vitro* mRNA transcripts prior to translation

Quality Control Statement

KACTUS manufactures this product according to GMP guidelines and performs stringent quality control testing before release. It is suitable for mRNA therapeutics and manufacturing of mRNA vaccines. Regulatory support documents are available. Please contact support@kactusbio.us for more information.

Quality Control Release Criteria

Assay	Criteria
Activity (probe incorporation)	≥ 10kU/mL
Purity (SEC-HPLC)	≥ 95%
Residual Endonuclease	Negative
Residual Exonuclease	Negative
Endotoxin	≤ 10EU/mL
Residual DNase	Negative
Residual RNase	Negative
Residual Protease	Negative
Residual Host Cell Protein	≤ 20ng/mg
Residual Host Cell DNA	≤ 100pg/mL
Residual Heavy Metal	≤ 10 ppm
Bioburden	≤ 1CFU/10mL

Protocol for mRNA Capping

1. Dilute purified RNA (up to 10µg) with Nuclease-free H_2O to 15µL in 1.5mL tube.
2. Incubate at 65°C for 5 min.
3. Place tube on ice for 5 min.
4. Prepare the following reaction mixture in the specified order in the table below.

Reagent	Quantity
Denatured RNA (step 3)	15µL
10X Capping Buffer	2µL
GTP (10mM)	1µL
SAM (2mM)	1µL
Vaccinia Capping Enzyme (10U/µL)	1µL

5. Incubate at 37°C for 30 minutes. For transcripts with known structured 5' ends, the reaction time can be extended to 60 min to improve capping efficiency.
6. RNA is now capped and ready for use in downstream applications. If the RNA needs a poly(A) tail, *E.coli* Poly (A) Polymerase (PLA-EE101) can be used.

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.

Protocol for Labeling 5' End of mRNA

1. Dilute purified RNA (up to 10µg) with Nuclease-free H_2O to 14µL in 1.5mL tube.
2. Incubate at 65°C for 5 minutes.
3. Place tube on ice for 5 minutes.
4. Prepare the following reaction mixture in the specified order in the table below.

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Reagent	Quantity
Denatured RNA	14µL
10X Capping Buffer	2µL
Labeled GTP (10mM)	2µL
SAM (2mM)	1µL
Vaccinia Capping Enzyme (10U/µL)	1µL

5. Incubate at 37°C for 30 minutes. For transcripts with known structured 5' ends, the reaction time can be extended to 60 min to improve capping efficiency.
6. RNA is now labeled and capped, and ready for use in downstream applications.

Notes

- The reaction can be scaled up as needed.
- RNA used for labeling 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 the 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.
- The labeling efficiency is impacted by the molar ratio of RNA: GTP. The total GTP concentration should be approximately 1-3 times the molar concentration of RNA in the reaction.