mRNA Cap 2´-O-Methyltransferase

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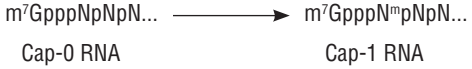
M0366S 003140316031

M0366S

2,000 units **50,000 U/ml** **Lot: 0031403**
RECOMBINANT **Store at –20°C** **Exp: 3/16**

Description: mRNA Cap 2´-O-Methyltransferase adds a methyl group at the 2´-O position of the first nucleotide adjacent to the cap structure at the 5´ end of the RNA. The enzyme utilizes S-adenosylmethionine (SAM) as a methyl donor to methylate capped RNA (cap-0) resulting in a cap-1 structure.



The cap-1 structure has been reported to enhance mRNA translation efficiency (1) and hence may help improve expression in mRNA transfection and microinjection experiments.

mRNA Cap 2´-O-Methyltransferase specifically requires RNA with an m^7GpppN cap as substrate. It cannot utilize RNA with pN, ppN, pppN or GpppN at the 5´ end (2,3). Capped RNA may be prepared via *in vitro* transcription using cap analog or through enzymatic capping using the Vaccinia Capping Enzyme (NEB #M2080).

The reagents provided in this pack can be used to methylate up to 400 µg of capped RNA.

Source: An *E. coli* strain that carries the gene for the Vaccinia mRNA Cap 2´-O-Methyltransferase.

Applications:

- 2´-O-methylation of capped mRNA for improved expression during microinjection and transfection experiments.

Supplied in: 20 mM Tris-HCl, pH 8.0, 100 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 0.1% Triton X-100 and 50% glycerol.

Reagents Supplied with Enzyme:

10X Capping Buffer	100 µl
SAM (32 mM)	100 µl

Reaction Conditions: 1X Capping Buffer. Incubate at 37°C.

1X Capping Buffer:
50 mM Tris-HCl, pH 8.0
5 mM KCl
1 mM $MgCl_2$
1 mM DTT

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.

Quality Control Assays

RNase Assay: Incubation of a 10 µl reaction containing 50 units of mRNA Cap 2´-O-Methyltransferase with 40 ng of 300-mer RNA transcript for 2 hours at 37°C resulted in less than 10% degradation of RNA as determined by denaturing PAGE analysis.

Exonuclease Activity: Incubation of a 50 µl reaction containing 50 units of mRNA Cap 2´-O-Methyltransferase with 1 µg of a mixture of single and double-stranded [^{3}H] *E. coli* DNA for 4 hours at 37°C released < 0.5% of the total radioactivity.

Endonuclease Activity: Incubation of a 50 µl reaction containing 50 units of mRNA Cap 2´-O-Methyltransferase with 1 µg of ϕX174 RF I DNA for 4 hours at 37°C produced less than 10% conversion to RF II as determined by agarose gel electrophoresis.

Protein Purity (SDS-PAGE): mRNA Cap 2´-O-Methyltransferase is > 99% pure as determined by SDS PAGE analysis using Coomassie staining.

Notes on Use
(READ PRIOR TO SETTING UP REACTION)

1. RNA prepared using *in vitro* transcription and cap analog should be purified prior to use and resuspended in nuclease-free water. EDTA and salts should not be present in the solution.

   mRNA Cap 2´-O-Methyltransferase may be directly added to a Vaccinia Capping System (NEB #M2080) reaction. RNA purification is not required in this case.
2. Heating the RNA at 65°C for 5 minutes prior to incubation with the enzyme removes secondary structure on the 5´ end of the transcript. Extend time to 10 minutes for transcripts with known highly structured 5´ ends.
3. SAM is unstable at pH 7–8, 37°C and should be mixed fresh prior to starting the reaction. We recommend determining how many reactions will be performed and diluting an aliquot of the 32 mM stock to 4 mM immediately before setting up the reactions. This "working stock" should be kept on ice to prevent degradation of SAM.

Protocols

2´-O-Methylation of Capped RNA
This protocol is designed to methylate up to 10 µg of capped RNA in a 20 µl reaction. Reaction size can be scaled up as needed.

1. Combine capped RNA and nuclease-free water in a final volume of 16 µl. (Refer to step 1 in the notes on use).
2. Heat at 65°C for 5 minutes (Refer to step 2 in the notes on use).
3. Place tube on ice for 5 minutes.
4. Add the following components in the order specified:

Denatured capped RNA (from above)	16.0 µl
10X Capping Buffer	2.0 µl
SAM (4 mM, dilute 32 mM stock to 4 mM)	1.0 µl
mRNA Cap 2´-O-Methyltransferase (50 U/µl)	1.0 µl
	20.0 µl

Note: Use of RNase Inhibitor is recommended to enhance stability of RNA in the reaction. Add 0.5 µl of RNase Inhibitor (e.g., Murine RNase Inhibitor NEB #M0314) during reaction set up. Subtract the additional volume from the amount of H_2O used in the reaction.

5. Incubate at 37°C for 60 minutes (For RNA less than 200 nt long increase incubation time to 2 hours)
6. Proceed with purification of the RNA (if required) for downstream applications.

One-Step Capping and 2´-O-Methylation
This protocol is designed to complete both capping and 2´-O-methylation in a single step. It involves incubating uncapped RNA with the Vaccinia Capping Enzyme (NEB #M2080, not included) and mRNA Cap 2´-O-Methyltransferase in the presence of GTP and SAM. The Vaccinia Capping Enzyme adds the cap at the 5´ end of the RNA followed by 2´-O-methylation by the methyltransferase. This protocol can synthesize up to 10 µg of cap-1 RNA in a 20 µl reaction. Reaction size can be scaled up as needed.

1. Combine uncapped RNA and nuclease-free water in a final volume of 14.0 µl. (Refer to step 1 in the notes on use).
2. Heat at 65°C for 5 minutes (Refer to step 2 in the notes on use).
3. Place tube on ice for 5 minutes
4. Add the following components in the order specified:

Denatured RNA (from above)	14.0 µl
10X Capping Buffer	2.0 µl
GTP (10 mM)	1.0 µl
SAM (4 mM, dilute 32 mM stock to 4 mM)	1.0 µl
Vaccinia Capping Enzyme (10 U/µl)	1.0 µl
mRNA Cap 2´-O-Methyltransferase (50 U/µl)	1.0 µl
	20.0 µl

Note: Use of RNase Inhibitor is recommended to enhance stability of RNA in the reaction. Add 0.5 µl of RNase Inhibitor (e.g., Murine RNase Inhibitor NEB #M0314) during reaction set up. Subtract the additional volume from the amount of H_2O used in the reaction.

5. Incubate at 37°C for 60 minutes (For RNA less than 200 nt long increase incubation time to 2 hours)
6. Proceed with purification of the RNA (if required) for downstream applications.

(see other side)

References:

1. Kuge, H. et al. (1998) *Nucleic Acids Res.* 26, 3208-3214.
2. Barbosa, E. and Moss, B. (1978) *J. Biol. Chem.* 253, 7698-7702.
3. Lockless, S.W. et al. (1998) *Biochemistry*. 37, 8564-8574.

Companion Products

Vaccinia Capping System
M2080S 400 units

RNase Inhibitor, Murine
M0314S 3,000 units
M0314L 15,000 units

RNase Inhibitor, Human Placenta
M0307S 2,000 units
M0307L 10,000 units

T7 High Yield RNA Synthesis Kit
E2040S 50 reactions

E. coli Poly(A) Polymerase
M0276S 100 units
M0276L 500 units

Ribonucleotide Solution Set
N0450S 10 µmol of each
N0450L 50 µmol of each