mRNA Cap 2´-O-Methyltransferase

2,000 units 50,000 U/ml Lot: 003130 RECOMBINANT Store at –20°C Exp: 10/15

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 RNA structure.

Applications:
- 2´-O-methylation of capped mRNA for improved expression during microinjection and transfection experiments.
- Enhance stability of RNA in the reaction.
- Add 0.5 µl of RNase Inhibitor (e.g., Murine RNase Inhibitor NEB #M2080) reaction. RNA purification is not required in this case.
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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.
2. 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.
3. 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.
4. 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.

Proteins

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 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
   - mRNA Cap 2´-O-Methyltransferase (50 U/µl) 1.0 µl
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
   - mRNA Cap 2´-O-Methyltransferase (50 U/µl) 1.0 µl
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:

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