mRNA Cap 2’-O-Methyltransferase

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 MgCl₂
- 1 mM DTT

Unit Definition: One unit is defined as the amount of enzyme required to methylate 10 pmol 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 [3H] 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 qX174 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.

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 reaction 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 7–9, 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

This protocol is designed to methylate up to 10 µg of capped RNA in a 20 µl reaction.

Endonuclease Activity: Incubation of a 50 µl reaction containing 50 units of mRNA Cap 2’-O-Methyltransferase with 1 µg of qX174 RF I DNA for 4 hours at 37°C produced less than 10% conversion to RF II as determined by agarose gel electrophoresis.

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 H₂O 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.

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References:

Companion Products

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