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# N6 Methylation of the Initiating Adenine of Capped RNA using mRNA Cap N6 Methyltransferase (m6A) (NEB #M0767)

## Overview

This is a general protocol designed to generate Cap-1 m6A RNAs by methylating the N6 position of the initiating adenine of capped RNA (i.e., Cap-0 or Cap-1 RNA) using mRNA Cap N6 Methyltransferase (m6A) (NEB #M0767). This method can enzymatically modify RNAs capped co-transcriptionally with cap analogs or post-transcriptionally by viral capping enzymes (FCE (M2081) / VCE (M2080)). The following reaction can be scaled up as needed.

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### Before starting

- Capped RNA prepared with *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.
- If using T7 RNA polymerase to synthesize uncapped RNA that initiates with adenine, the T7 Promoter class II is recommended, with the first three transcribed nucleotides being AGA: 5'- TAATACGACTCACTATTAGGA 3'. T7 RNA polymerase starts transcription at the underlined A (+1 position) in the sequence.

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## Protocol



### Precautions:

- Wearing gloves and using nuclease-free tubes and reagents is strongly recommended to avoid RNase contamination.
- SAM is unstable at pH 7-8 at 37°C. Add SAM immediately before starting the reaction.

1. Combine up to 180 µg capped RNA and nuclease-free water to a final volume of 42 µl.  
*Optional:* Heat at 65°C for 5 minutes to reduce RNA secondary structure prior to capping. This step is not necessary for all substrates.
2. Place the tube on ice.
3. Set up the following reaction on ice in the following order specified:

COMPONENT	50 $\mu$ l REACTION	FINAL AMOUNT
Capped RNA	42 $\mu$ l	to 180 $\mu$ g
10X Capping Buffer	5 $\mu$ l	1X
32 mM SAM	1 $\mu$ l	0.64 mM
mRNA Cap N6 Methyltransferase (m6A)	2 $\mu$ l	50 units

4. Incubate at 37°C for 60 minutes.
5. Proceed with [purification of RNA](#) (if required) for downstream applications.



## General Guidelines

1. For highly structured substrates and RNAs shorter than 1000 nt, more enzyme and/or longer reaction times may be required to yield complete capping.
2. The standard T7 promoter can be used if the RNA was capped with a cap analog that begins with adenine.

## Related Resources

- [RNA Capping](#)
- [RNA Extraction and Purification](#)