For life science research only.
Not for use in diagnostic procedures.

2’-O-Methyltransferase

Version: 02
Content Version: August 2025

Cat. No. 10 424 346 001 500 µg

Store the product at −15 to −25°C.

1. General Information

1.1. Contents

Vial / bottle	Cap	Label	Function / description	Content
1	colorless	2′-O-Methyltransferase	▪ Activity: ≥45 U/mL. ▪ Protein concentration: 0.8 to 1.2 mg/mL. ▪ Buffered solution with 50% (v/v) glycerol and stabilizers, pH 7.5.	1 vial, 667 µL
2	green	2′-O-Methyltransferase, Reaction Buffer, 10x conc.	Composition: 500 mM Tris-HCl, 50 mM KCl, 10 mM $MgCl_2$, 10 mM dithiothreitol (DTT), pH 8.0.	2 vials, 500 µL each
3	yellow	2′-O-Methyltransferase, S-Adenosylmethionine (SAM)	Supplied as 40 mM aqueous stock solution, pH <4.0.	1 vial, 25 µL

1.2. Storage and Stability

Storage Conditions (Product)

When stored at −15 to −25°C, the product is stable through the expiration date printed on the label.

Vial / bottle	Cap	Label	Storage
1	colorless	2′-O-Methyltransferase	Store at −15 to −25°C.
2	green	Reaction Buffer, 10x conc.	
3	yellow	S-Adenosylmethionine (SAM)	

1.3. Additional Equipment and Reagent required

Standard laboratory equipment

- Nuclease-free pipette tips
- 1.5 mL RNase-free microcentrifuge tubes to prepare master mixes and dilutions
- To minimize risk of RNase contamination, autoclave all vessels
- Centrifuge with swinging-bucket rotor

Wear gloves at all times.

For *in vitro* transcription

- T7 RNA Polymerase, rec.*
  500 mM $MgCl_2$ and 10x Reaction Buffer: 400 mM Tris-HCl (pH 8.0), 20 mM spermidine, and 100 mM dithiothreitol (DTT) are supplied with each of the RNA Polymerases.
- 100 mM Nucleotides ATP*, GTP*, CTP*, UTP* (or Pseudo-UTP* or N^1-Methyl-Pseudo-UTP*)
- DNase I, rec.*
- Pyrophosphatase, rec.*
- RNase Inhibitor*
- DNA template
- RNase-free water

For capping of mRNA

- Water, PCR Grade*
- Template RNA
- mRNA Guanylyltransferase*
- RNase Inhibitor* (optional)

1.4. Application

2'-O-Methyltransferase can be used for the enzymatic methylation of a 7-methylguanylate cap (Cap-0) structure, generating the Cap-1 structure. The Cap-1 structure is formed from m^7G-ppp-RNA (RNA with Cap-0 structure, m^7G-ppp-RNA, from the action of mRNA Guanylyltransferase) by catalyzing a 2′-O-methylation in the ribose of the first transcribed nucleotide, consuming S-Adenosylmethionine (SAM) in the process.
5′-cap structures of mRNA are key to transcript stability, supporting translation and function, and reducing immunogenicity and degradation to achieve stable protein expression.

Off-the-shelf kits for enzymatic mRNA processing are commonly used in research and early stages of development. However, such kits reduce flexibility to optimize reagents for different mRNA molecules based on composition, length, and sequence. Furthermore, a switch to high quality reagents is often needed for *in vivo* experiments with small animal models. To support your needs, Roche provides small pack sizes of high quality individual reagents, developed in a fit-for-purpose (FFP)[1] and animal-origin-free (AOF) format by Roche CustomBiotech.

The supplied Reaction Buffer, 10x conc. (Vial 2) and S-Adenosylmethionine (SAM) (Vial 3) are not tested for fit-for-purpose (FFP) and animal-origin-free (AOF).

For Bulk reagents, contact Roche CustomBiotech https://go.roche.com/cbcontact and/or https://custombiotech.roche.com/mrna.

[1] Read more about the Roche fit-for-purpose mRNA portfolio: https://go.roche.com/mrnafitforpurpose.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

For RNA input material, use:
- High quality intact RNA in nuclease-free water.
- RNA with a low-salt content, especially when using high amounts in the enzymatic capping.
- No EDTA or other chelator additives that can complex Mg^{2+} ions.

When coming from an *in vitro* transcription, purify the RNA before further use.

Only RNA substrates with a Cap-0 structure are accepted by the enzyme and the first nucleobase of the transcript must be adenine or guanine (m^7G-ppp-A-RNA or m^7G-ppp-G-RNA). RNA generated by T7-, or other RNA polymerases must be modified by the action of a Cap-0-generating enzyme, such as mRNA Guanylyltransferase, before using 2'-O-Methyltransferase or can be modified in a one-step reaction.

General Considerations

Precautions

To minimize the risk of RNase contamination:
- Autoclave all vessels and pipette tips that will be used in the RNA capping reaction.
- Wear gloves at all times.
- Keep reagents on ice after thawing.
- Briefly centrifuge all reagents before beginning the procedure.

Safety Information

Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation or take appropriate measures, according to local safety regulations.
- Do not eat, drink, or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats, and eye protection when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online at www.sigmaaldrich.com, or upon request from www.sigma-aldrich.com/techservice.

Working Solution

Due to the small volume of reagents required for each RNA capping reaction, prepare a larger amount of the master mix, centrifuge briefly, and aliquot into sample reaction tubes before adding the RNA sample. Preparation of this master mix eliminates the need to repeatedly pipette small volumes, resulting in increased consistency between samples.

SAM is provided as a 40 mM stock solution and should be diluted with Water, PCR Grade to a 2 mM working solution.

This solution must be prepared fresh, before each capping reaction and used immediately after dilution. Diluted S-Adenosylmethionine (SAM) is known for its instability at physiological or alkaline pH, particularly at elevated temperatures. To ensure consistent results, always prepare freshly diluted SAM working solutions.

2.2. Protocols

Starting guidelines for the capping reaction setup

Here we provide a guideline for a starting protocol that must be optimized according to the individual RNA template and process and reaction conditions. This generic protocol is designed to cap 10 µg of RNA (1 kb transcript, precapped with mRNA Guanylyltransferase) in a 20 µL reaction using low-volume microfuge tubes (1.5 mL). The reaction size is adjustable and can be scaled according to experimental requirements. The product provides sufficient reagents to perform at least 500 reactions at this standard scale.
When optimizing or adapting the reaction to meet experimental or process requirements, begin by adjusting the amounts of enzyme and RNA.

- Start by reducing the enzyme concentration and/or modifying the enzyme-to-RNA ratio.
- It is essential to maintain SAM in molecular excess relative to the template RNA to prevent substrate limitation.
- The amount of S-Adenosylhomocysteine (SAH) produced by the methyltransferase activity directly corresponds to the amount of capped RNA.

See section, **Working Solution** *for information on preparing the master mix.*
Wear gloves at all times.

Follow the procedure below to prepare one 20 µL standard reaction.

1. Thaw the solutions and store on ice.

2. Briefly vortex and centrifuge all reagents before setting up the reactions.

3. In a 1.5 mL reaction tube on ice, add the following components in the order listed below:

Reagent	Volume [µL]	Final conc.
Reaction Buffer, 10x conc. (Vial 2) 500 mM Tris-HCl, 50 mM KCl, 10 mM $MgCl_2$, 10 mM DTT, pH 8.0	2.0	1x
2′-O-Methyltransferase (Vial 1)	1.0	40 – 60 µg/mL
S-Adenosylmethionine (SAM), 2 mM working solution	1.0	100 µM
Template RNA[1]	Variable, for example, 10 µg	–
Water, PCR Grade	X µL (make up the volume to 20 µL)	–
Total volume	**20 µL**	

4. Gently mix and centrifuge the mixture to collect the sample at the bottom of the reaction tube.

5. Incubate the reaction at +37°C for 30 minutes.

   Adding 0.5 µL RNase Inhibitor per 20 µL will protect the RNA from degradation. Deduct the extra volume from the water in the reaction.

6. The RNA now possesses a Cap-1 structure and is ready for downstream applications.

   Some applications may require the RNA to be purified prior to use.

[1] Incubating RNA at +65°C for 5 to 10 minutes prior to the reaction removes secondary structures and can help to improve capping efficiency.

One-step Cap-1 addition with mRNA Guanylyltransferase

To generate RNA with the Cap-1 structure in a single process step, mRNA Guanylyltransferase and 2′-O-Methyltransferase can be utilized in a one step reaction. This eliminates the need for additional purification steps, reduces hands-on time, and simplifies the procedure.
The composition of both the Reaction Buffer concentrates and SAM solutions across both products are identical.
To adapt to a one-step capping reaction, start with the generic protocol for mRNA Guanylyltransferase, see Instructions for Use of mRNA Guanylyltransferase. Incorporate the recommended volume of 2′-O-Methyltransferase according to its generic protocol above. Double the amount of SAM, and extend the reaction time to 60 minutes. The progress and yield of capping, as well as specific requirements, will depend on several factors: the length of the template RNA, its accessibility and secondary structure, the presence of other compounds or residues, such as salts, and overall reaction conditions.

2.3. Parameters

EC-Number

EC 2.1.1.57

Molecular Weight

40 kDa

Unit Definition

One unit of 2′-O-Methyltransferase is the amount that produces 1 nmol of the capped product (Cap-1 structure) from a 4 nucleotide long RNA substrate (with Cap-0 structure) per minute at +37°C.

3. Additional Information on this Product

3.1. Quality Control

For lot-specific certificates of analysis, see section **Contact and Support**.

4. Supplementary Information

4.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

4.2. Changes to previous version

Editorial changes.
Updated Ordering Information.

4.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
DNase I, rec.	10 KU	10 428 376 001
N¹-Methyl-Pseudo-UTP	10 µmol	10 427 922 001
mRNA Guanylyltransferase	250 µg	10 424 338 001
T7 RNA Polymerase, rec.	10 KU	10 393 351 001
	30 KU	10 393 343 001
Pseudo-UTP	10 µmol	10 427 990 001
RNase Inhibitor	20 KU	10 428 007 001
Pyrophosphatase, rec.	60 U	10 428 392 001
GTP	25 µmol	10 436 034 001
UTP	25 µmol	10 436 468 001
ATP	25 µmol	10 436 484 001
CTP	25 µmol	10 436 492 001

4.4. Trademarks

All product names and trademarks are the property of their respective owners.

4.5. License Disclaimer

For patent license limitations for individual products please refer to:
Product Disclaimers.

4.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

4.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

4.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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