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

Pseudo-UTP

Version: 02
Content Version: August 2025

Cat. No. 10 427 990 001 10 µmol

Store the product at −15 to −25°C.

1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Content
1	Pseudo-UTP	Supplied as 100 mM solution.	1 vial, 10 µmol

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	Label	Storage
1	Pseudo-UTP	Store at −15 to −25°C.

1.3. Additional Equipment and Reagent required

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 N^1-Methyl-Pseudo-UTP*)
- DNase I, rec.*
- Pyrophosphatase, rec.*
- RNase Inhibitor*
- DNA template
- RNase-free water

For capping of mRNA

Co-transcription can be performed using cap analogs during the IVT reaction; Post-transcriptional enzymatic capping of mRNA is done as an additional step after IVT.

- Cap analog
- mRNA Guanylyltransferase*
- 2′-O-Methyltransferase*

1.4. Application

Pseudo-UTP is used as a substitute for conventional UTP in *in vitro* transcription reactions. This modified UTP generates a modified RNA with enhanced protein expression and reduced unwanted immunogenicity against the mRNA molecule itself. Although the exact mechanisms, by which modified ribonucleotides influence mRNA immunogenicity or protein production have not been defined, they exert their influence by altering one or more of the following properties of mRNA:

Reduced mRNA immunogenicity

- Altering RNA recognition by intracellular receptors of the immune defense system (**Anderson BR, et al, 2010**).
- Altering mRNA secondary structure and thereby influencing RNA-receptor interactions (**Durbin AF, et al, 2016; Karikó K, et al, 2005**).
- Altering antisense transcript synthesis by decreasing the "self-priming" effect of RNA polymerase, which reduces dsRNA (**Vaidyanathan S, et al, 2018**).

Increased protein production

- By regulating functional mRNA half-life (**Mauger DM, et al, 2019**).
- Via increased translation (**Li B, et al, 2016; Svitkin YV, et al, 2017**).

Off-the-shelf kits for *in vitro* transcription (IVT) of mRNA 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 template. 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.

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

Use plasmid DNA or purified PCR template containing the sequence of interest for *in vitro* transcription reactions.

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.

2.2. Protocols

Starting guidelines for *in vitro* transcription (IVT) reaction setup

In vitro synthesis of single-stranded mRNA molecules is a widely used laboratory procedure that is central to RNA research and therapeutic development. Here we provide a general guideline for a starting protocol for a 20 µL IVT reaction.

The protocol must be optimized according to specific template constructs and their use. Optimizing the IVT reaction helps improve yield and reduces dsRNA formation.

1. Pipette the following reaction master mix for a 20 µL reaction.

   If volume or the amount are not specified in the following table, use the recommendations in the Final conc. column to calculate the volumes.

Reagent	Volume/amount [per 20 µL reaction]	Final conc. [per 20 µL reaction volume]
Reaction Buffer, 10x conc. (supplied with RNA Polymerase) 400 mM Tris-HCl (pH 8.0), 20 mM spermidine, and 100 mM DTT	2 µL	1x
T7 RNA Polymerase, rec. (1)	–	100 U/µL
100 mM nucleotides ATP, GTP, CTP, UTP (alternatively, modified nucleotide Pseudo-UTP or N1-Methyl-Pseudo-UTP)	–	10 mM each
500 mM $MgCl_2$ (supplied with RNA Polymerase)(2)	–	30 mM
Cap analog(3)	–	4 mM
RNase Inhibitor(4)	–	1 U/µL
Pyrophosphatase, rec.(5)	–	0.002 U/µL
Template DNA	250 ng of plasmid DNA or 100 ng of PCR template	–
RNase-free water	X µL (make up the volume to 20 µL)	–
Total volume	**20 µL**	

2. Make up the reaction volume to 20 µL with RNase-free water.

3. Incubate the IVT reactions at +37°C for 2 hours.

4. After incubation, treat the IVT reaction with DNase I, rec. at a concentration of 0.4 U/µL of the reaction volume for 30 minutes to 1 hour at +37°C, in order to remove the template DNA prior to purification.

(1) To improve yield, titrate the template and T7 RNA Polymerase, rec. concentrations.
(2) The concentration of nucleotides and Mg^{2+} ions is crucial to achieve a good yield and low dsRNA.
(3) Optional, only for co-transcriptional capping. 5′-cap can also be added post-transcriptionally using capping enzymes.
(4) Appropriate quantities of RNase Inhibitor should be added to protect RNA from degradation.
(5) Addition of Pyrophosphatase, rec. removes any inhibitory pyrophosphates that may be generated during the IVT reaction.

The capping of mRNA at the 5′ end enhances its stability, supports efficient translation, and reduces immunogenicity. If no cap analogs were used during IVT, use the capping enzymes mRNA Guanylyltransferase and 2′-O-Methyltransferase for post-transcriptional capping in a separate step

3. Additional Information on this Product

3.1. References

- Svitkin YV, et al. N1-methyl-pseudouridine in mRNA enhances translation through eIF2α-dependent and independent mechanisms by increasing ribosome density. Nucleic Acids Res. 2017;45:6023-6036.
- Li B, Luo X, Dong Y. Effects of chemically modified messenger RNA on protein expression. Chem. 2016;27:849-853.
- Mauger DM, Cabral BJ, Presnyak V, et al. mRNA structure regulates protein expression through changes in functional half-life. Proc Natl Acad Sci USA. 2019;116:24075-24083.
- Vaidyanathan S, Azizian KT, Haque AKMA, et al. Uridine depletion and chemical modification increase Cas9 mRNA activity and reduce immunogenicity without HPLC purification. Mol Ther Nucleic Acids. 2018;12:530-542.
- Karikó K, Buckstein M, Ni H, et al. Suppression of RNA recognition by toll-like receptors: The impact of nucleoside modification and the evolutionary origin of RNA. Immunity. 2005;23:165-175.
- Durbin AF, Wang C, Marcotrigiano J, Gehrke L. RNAs containing modified nucleotides fail to trigger RIG-I conformational changes for innate immune signalling. mBio. 2016;7:e00833-16.
- Anderson BR, Muramatsu H, Nallagatla SR, et al. Incorporation of pseudouridine into mRNA enhances translation by diminishing PKR activation. Nucleic Acids Res. 2010;38:5884-5892.

3.2. 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		
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
T7 RNA Polymerase, rec.	10 KU	10 393 351 001
	30 KU	10 393 343 001
N^1-Methyl-Pseudo-UTP	10 µmol	10 427 922 001
DNase I, rec.	10 KU	10 428 376 001
mRNA Guanylyltransferase	250 µg	10 424 338 001
2'-O-Methyltransferase	500 µg	10 424 346 001

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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