

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



T7 RNA Polymerase, rec.

 **Version: 02**

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Cat. No. 10 393 351 001 10 KU

Cat. No. 10 393 343 001 30 KU

Store the product at -15 to -25°C .

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1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Catalog number	Content
1	T7 RNA Polymerase, rec.	Enzyme supplied RNase-free in glycerol.	10 393 351 001	1 vial, 12 µL (≥10,000 units)
			10 393 343 001	3 vials, 3 × 12 µL each (≥30,000 units)
2	T7 RNA Polymerase, rec., Reaction Buffer, 10x conc.	Contains 400 mM Tris-HCl (pH 8.0), 20 mM spermidine, and 100 mM dithiothreitol (DTT).	10 393 351 001	1 vial, 300 µL
			10 393 343 001	1 vial, 300 µL
3	T7 RNA Polymerase, rec., MgCl ₂ , 500 mM	To adjust MgCl ₂ concentration.	10 393 351 001	1 vial, 300 µL
			10 393 343 001	1 vial, 300 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	Label	Storage
1	T7 RNA Polymerase, rec.	Store at –15 to –25°C.
2	Reaction Buffer, 10x conc.	
3	MgCl ₂ , 500 mM	

1.3. Additional Equipment and Reagent required

For *in vitro* transcription

- 100 mM nucleotides ATP*, GTP*, CTP*, UTP* (or Pseudo-UTP* or N¹-Methyl-Pseudo-UTP*)
- RNase Inhibitor*
- Pyrophosphatase, rec.*
- DNase I, rec.*
- 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*

2. How to Use this Product

1.4. Application

T7 RNA Polymerase, rec. is the key enzyme for *in vitro* transcription of mRNA. It generates mRNA from double-stranded DNA templates carrying the T7 promoter sequence and efficiently incorporates standard or modified ribonucleoside triphosphates (NTPs) into mRNA transcripts.

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.

i The supplied Reaction Buffer, 10x conc. (Vial 2) and MgCl₂, 500 mM (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

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 μ L IVT reaction.

i 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 μ L reaction.

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

2 Make up the reaction volume to 20 μ L 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/ μ L 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 Mg²⁺ 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.

i 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. 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	
 <i>Information Note: Additional information about the current topic or procedure.</i>	
 Important Note: Information critical to the success of the current procedure or use of the product.	
 etc.	Stages in a process that usually occur in the order listed.
 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		
UTP	25 µmol	10 436 468 001
RNase Inhibitor	20 KU	10 428 007 001
Pyrophosphatase, rec.	60 U	10 428 392 001
GTP	25 µmol	10 436 034 001
CTP	25 µmol	10 436 492 001
ATP	25 µmol	10 436 484 001
Pseudo-UTP	10 µmol	10 427 990 001
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.

