T7 RNA Synthesis Kit

User Manual

Cat. No. **PC035 (50 reactions)**
Cat. No. **PC036 (100 reactions)**

User Manual

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T7 RNA Synthesis Kit

I. Description

T7 RNA Synthesis Kit is designed for *in vitro* transcription of large amounts of RNA, provides with T7 RNA polymerase mix and four individually available nucleotides. The kit can also be used to synthesise capped RNA by adding cap analogues; or to synthesise modified RNA by replacing with modified UTP according to experimental requirements. The kit also contains LiCl Solution for removing free nucleotides, enzymes and most of the template from the transcription product to obtain coarsely purified RNA.

■ Application

Synthesis of large amounts of various RNAs, including but not limited to:

1. mRNA for vaccine or gene expression
2. precursors of circular RNA
3. labeled RNA probes
4. Modified RNA: prepared by admixing modified NTP (e.g., aminoallyl-, biotin-, fluorescein-, digoxin-NTP)

II. Contents and Storage

Store all components at -20℃, except LiCl solution at 4℃ (stable for at least 12 months). Avoid repeated freezing/ thawing.

Cat. No.	Contents	Part No.	Quantity
PC035	T7 RNA Polymerase Mix	PC035-01	100 μL
	10× IVT Buffer	PC035-02	100 μL
	ATP Solution (100 mM)	PC035-03	100 μL
	CTP Solution (100 mM)	PC035-04	100 μL
	GTP Solution (100 mM)	PC035-05	100 μL
	UTP Solution (100 mM)	PC035-06	100 μL
	Control DNA Template (0.5 ng/μL)	PC035-07	10 μL
	LiCl Solution	PC035-08	1.2 mL
	DTT (100 mM)	PC035-09	50 μL
	ddH_2O (DNase / RNase Free)	PC035-10	2×1 mL

III. Procedures

■ DNA Template Preparation:

Linearized plasmid DNA, PCR products or synthetic DNA oligonucleotides can be used as templates for *in vitro* transcription with the kit, provided that they contain a double-stranded T7 promoter region upstream of the sequence to be transcribed.

Linearized plasmid DNA:

High purity and fully linearized plasmids are necessary to obtain high yields and intact RNA. Direct use of unlinearized plasmids significantly reduce yields and contains more excessively long by-products. In order to prepare large amounts of RNA of a specific length, the plasmid must be fully linearized. Quality control can be performed by electrophoresis to verify the singularity and integrity of the linearized DNA. The linearized plasmid should have blunt ends or 5´-overhangs and need to be purified before downstream transcription experiments.

PCR product:

The PCR product with the T7 promoter can be used as a template for in vitro transcription. Purification of the PCR product is required for downstream transcription.

Synthetic DNA oligonucleotides:

Synthetic double- or single-stranded DNA with a double-stranded T7 promoter sequence can be used as a template for in vitro transcription.

■ RNA Synthesis:

1. Assemble the reaction on ice in the following order:

Reagent	Volume	Final concentration
ddH_2O (DNase / RNase Free)	Total 20 μL	
10× IVT Buffer	2 μL	1×
ATP/CTP/GTP/UTP Solution	2 μL each	10 mM
DNA Template		1 μg
T7 RNA Polymerse Mix	2 μL	
DTT	1	5 mM

2. Mix the reaction solution well. Spin down briefly.
3. Incubate at 37℃ for 2 hours.

■ Purification of Synthesized RNA:

The kit includes LiCl solution for quick recovery of the synthesized RNA. LiCl precipitation of RNA is effective in removing enzymes and unincorporated NTPs.

LiCl precipitation

1. Add equal volumes of RNase-free ddH2O and equal volume of LiCl solution, mix well, and spin down briefly.
2. Incubate at -20℃ for more than 1 h.
3. Centrifuge at 12,000 rpm for 15 min at 4℃ to precipitate RNA. Discard the supernatant, spin down briefly and aspirate with a small tip.
4. Add 500 µL of pre-cooled 70% ethanol to wash the pellet without disturbing it.
5. Centrifuge at 12,000 rpm for 5 min at 4℃, discard supernatant, spin down briefly and aspirate with a small tip.

Optional: Use **DNase I (*Cat. No.* PC024)** to remove template DNA

Add 12 µL of 10× DNase I Buffer and 2 µL of DNase, then add RNase-free ddH2O to bring the total volume to 120 µL. Mix the reaction solution thoroughly and incubate at 37℃ for 10 min. After the reaction, add 60 µL of LiCl solution and repeat the **LiCl precipitation** steps outlined above.

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■ **Note:**

a. T7 RNA Polymerase Mix is susceptible to oxidation, and its performance can be assured by adding DTT to each preparation. Do not leave DTT at room temperature or with the lid open for a long time.
b. Incomplete linearisation of the template reduces the yield and purity of the product.
c. Alcohol residues can greatly affect IVT efficiency, please ensure that the recovered template 260/230 > 2.2. When recovering by precipitation, ensure complete removal of the supernatant after each centrifugation. When using spin columns, ensure an additional spin is performed after the washing step.
d. The amount of template added can be reduced when using purified PCR products as template.
e. When the transcription product length is <300 nt, the reaction time can be extended to 4-16 h and the amount of DNA template can be increased to 2 µg in order to increase the yield of short transcripts.
f. When the DNA template concentration is sufficiently high, further increases the template concentration only affects the reaction speed, not the yield. Set up multiple reaction if you need to increase the yield.
g. The Control DNA Template is an enzymatically linearized plasmid template encoding Gluc, which can be used for transfection after purification of the transcript. This template has a T7 promoter followed by an AG start, and is compatible with cap analogues that require an AG start.

h. If the refrigerator temperature is not low enough for LiCl to precipitate RNA at -20℃, the precipitation effect will be reduced. It is recommended to place the product in an alcohol bath in a closed container at -20℃ or extend the incubation time in the refrigerator. Do not place at -80℃, as freezing will affect the precipitation effect.

i. When recovering RNA with LiCl, it is important to make sure that the supernatant is completely removed before adding 70% ethanol, otherwise NTP will be precipitated, causing the measured concentration to be falsely high.

j. The removal of the template with DNase I needs to be carried out after LiCl purification in order to make the DNA template undetectable by qPCR. If only crude purification is required, purification with LiCl is sufficient, as LiCl is not effective in precipitating DNA and protein. The bands of the DNA template are not visible after LiCl purification.

k. LiCl precipitation is very dependent on RNA concentration, therefore, RNA concentration should be kept above 500 ng/μL for multiple purifications.

l. When purifying RNA with LiCl, drying at 37℃ for 5 min with the lid open is for 20 μL of system product. If the reaction system is scaled up, the drying time should be extended to 10 min or changed to 1 min at 65℃ with the lid open. Be careful not to over-dry. After the specified drying time, the remaining liquid is generally water.

IV. Limited Use License and Warranty

Limited Use License

The following terms and conditions apply to the use of T7 RNA Synthesis Kit (the Product). If the terms and conditions are not acceptable, the Product in its entirety must be returned to GeneCopoeia within 5 calendar days. A limited End-User license is granted to the purchaser of the Product. The Product shall be used by the purchaser for internal research purposes only. The Product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use. The Product must not be resold, repackaged, or modified for resale, or used to manufacture commercial products without prior written consent from GeneCopoeia. This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research. Use of any part of the Product constitutes acceptance of the above terms.

Limited Warranty

GeneCopoeia warrants that the Product meets the specifications described in the accompanying Product Datasheet. If it is proven to the satisfaction of GeneCopoeia that the Product fails to meet these specifications, GeneCopoeia will replace the Product. In the event a replacement cannot be provided, GeneCopoeia will provide the purchaser with a refund. This limited warranty shall not extend to anyone other than the original purchaser of the Product. Notice of nonconforming products must be made to GeneCopoeia within 30 days of receipt of the Product. GeneCopoeia's liability is expressly limited to replacement of Product, or a refund limited to the actual purchase price. GeneCopoeia's liability does not extend to any damages arising from use or improper use of the Product, or losses associated with the use of additional materials or reagents. This limited warranty is the sole and exclusive warranty. GeneCopoeia does not provide any other warranties of any kind, expressed or implied, including the merchantability or fitness of the Product for a particular purpose.

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